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THE SIGNIFICANCE OF GLYCOSURIA*

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BECAUSE of the ease with which the presence of a reducing substance may be demonstrated in the urine, glycosuria is a very important and very frequent finding in the careful examination of patients. The interpretation of this symptom is not, however, always simple. An improper diagnosis is not rare, to the great discomfort or detriment of the patient. On the one hand, in the absence of a clear diabetic symptomatology, the physician too frequently assures him that he has a little sugar in his urine which is perfectly innocuous. Eventually the patient meets with catastrophies that are inevitable in the uncontrolled diabetic. On the other hand, the diagnosis of diabetes is made in many cases of nondiabetic glycosuria, with the restricted diet and unfavorable prognosis attached to that disease.

Glycosuria is not, of course, a disease any more than is fever or headache or jaundice. Like fever, headache and jaundice, it is a symptom and like them, may be due to any one of a number of diseases. It is the purpose of this communication to attempt to differentiate between the more common conditions in which sugar is found in the urine. While it is probable that all urine contains glucose, the word glycosuria is here used only in the clinical sense, namely to designate that condition in which it is sufficient in amount to reduce the ordinary laboratory reagents.

A word concerning the identification of glucose in the urine is not out of place. All reducing substances in the urine are not glucose. The most common example of this and the one most frequently leading physicians astray is the lactosuria of the pregnant or lactating woman. In such a case, the presence of a reducing substance in the urine, in the absence of diabetic

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symptoms and especially with a normal sugar content of the blood should make one suspect that he is dealing with lactose rather than glucose. Because of the well-known fact that pregnancy is not only a serious complication of diabetes but may be an important factor in precipitating the disease, the distinction between the two kinds of sugar is essential. For practical purposes it is sufficient to realize that the glucose is rapidly fermented by yeast, with the production of gas, whereas most of the urines containing reducing substances other than glucose are fermented slowly or not at all. More technical studies of polaroscopic activity and the osazone crystals are only occasionally required. The following case illustrates this:

CASE No. 1. A young woman, seventeen years of age, who had been normally delivered of a baby three weeks previously, was admitted to the hospital with a letter from her family physician, from which the following is quoted:

"This is to introduce Mrs. N. C., who is suffering from a very acute diabetes mellitus, whom I am sending you for treatment. I consider this a very grave case and believe she must receive treatment at once . . . ."

No history of symptoms suggestive of diabetes mellitus could be elicited. She had weaned her baby just before leaving home. Physical examination showed nothing of importance except evidence of her puerperal status. The blood sugar was normal. The urine reduced Fehling's and Benedict's solution, and with Benedict's quantitative solution gave a reaction corresponding to 3 to 6 gms. glucose in the first four twenty-four-hour urine specimens. There was no production of gas with yeast. In spite of the fact that she was on a "house diet," with no restriction of carbohydrates, her urine was negative during the following seven days. It is obvious that the condition was lactosuria rather than glycosuria, and that it cleared when she stopped nursing the baby.

In the normal subject the kidney is able to prevent the passage into the urine of any significant part of the tenth of one per cent of glucose that is in the blood. This balance may become upset by an abnormality of either part of the system, the kidney or the blood. If the kidney loses part of its ability to prevent the escape of sugar into the urine, or if the amount of sugar in the blood rises above the ability of the kidney to retain it, glycosuria occurs. The situation may roughly be compared to that of a river with a mill-dam. The water from the upper levels will overflow the dam if the sluice gate be opened or if a freshet increases the amount of water behind the dam. So glycosuria occurs if the "renal threshold" to glucose be lowered, or if the glycemia be raised above the renal threshold. Glycosurias, then, may be divided into two groups; those in which the blood sugar is normal or diminished and those in which it is elevated.

Glycosuria without elevation of the blood sugar is produced experimentally by a number of drugs, of which the most important is phloridzen. The administration of phloridzen to dogs and men has added materially to our knowledge of the intermediary metabolism, but this condition is not, of course, met in patients. Clinically, one sees glycosuria without hyperglycemia in the not rare condition known as renal glycosuria, or improperly, renal diabetes. Since this disease is apparently innocuous and calls for no treatment, it is important to distinguish it clearly from diabetes mellitus. The criteria on which the diagnosis rests are four: (1) specimens of urine and of blood, collected simultaneously, must demonstrate glycosuria with a normal

or depressed glycemia; (2) there must be no symptoms of diabetes mellitus; (3) the glycosuria must not parallel the carbohydrate content of the diet; and (4) the patient must not later develop true diabetes mellitus. Following is an example of this anomaly:

CASE No. 2. A negress, twenty-four years of age, entered the hospital for examination of her chest during convalescence from a pneumonia she had suffered eight months before in another city. Because of vague thoracic pains and night sweats, tuberculosis or empyema had been suspected, but no evidence could be obtained by physical or laboratory examination including x-ray, that there was any pulmonary disease. There was no history of diabetic symptoms. During the routine examination, her urine was found to reduce Benedict's solution, and further studies by fermentation, polaroscope and phenolhydrazine proved that the reducing substance was glucose. Her blood sugar on numerous occasions was between 0.043 per cent and 0.100 per cent and heavy glycosuria was always found. Oral administration of 100 gms. glucose was followed by a maximum glycemia of 0.092 per cent. She was under observation for nearly four months. During this time she was on five dietetic regimes, including fasting (six days), a 900 calory diabetic diet, a 2400 calory diabetic diet, the latter with 100 gms. extra glucose daily and an unrestricted "house diet." On all these varying regimes her average glucose output was between 50 and 60 gms. daily. She was sent home on an unrestricted diet, and reported 14 months later that she felt entirely well, though there was still a heavy glycosuria.

Of the glycosurias accompanied by an elevated blood sugar, the simplest is due to the excessive ingestion of sugar by a normal subject. Glycosuria may be caused in anyone by this method and cane sugar seems to be nearly as effective as glucose. Glucose is poured into the blood from the intestinal tract at more rapid rate than it is removed, the blood sugar rises until the renal threshold is passed, and glycosuria results. On the other hand, starch is slowly absorbed and never causes glycosuria in the normal subject. The term "alimentary glycosuria" should, in my opinion, be restricted to that group in which sugar appears in urine of the normal subject because of an excessive sugar intake. If it is the result of an excessive starch intake or if it follows the administration of amounts of sugar that would be tolerated by the normal subject it is not alimentary, but is due to some defect in the metabolism or mobilization of carbohydrate. Normally one may expect a fasting adult to take 100 gms. glucose in 40 per cent solution without glycosuria. If such a test meal causes glycosuria, the subject is not normal, and it is unjust to him to diagnose alimentary glycosuria. If, on the other hand, glycosuria follows the administration of 250 gms. of glucose, the glycosuria may be alimentary, and not due to any underlying disease.

This type of glycosuria is of clinical importance chiefly because it is not rarely found during routine examinations of large groups of patients or normal subjects. For example we see it frequently in students who come to the Medical Out-Patient Department with some nondiabetic complaint. A history is usually obtained of a fairly liberal lunch with pastry, dessert and coffee with sugar, followed by half a pound or even a pound of candy. It is not surprising that glucose appears in the urine. Similar cases are found in any large group of routine urinalyses, and the diagnosis of serious disease is not justified. If the subject is told to continue his regular diet, omit the candy and return for repeated examinations, his urine is found to be normal.

It should be emphasized that alimentary glycosuria is a normal phenomenon, associated with an excessive sugar intake; if there is any disturbance in the carbohydrate metabolism, some other diagnosis must be made.

Intense emotion seems to be a common cause of glycosuria and is associated with hyperglycemia. It has been shown that a large fraction of a group of students taking an examination may have glucose in the urine. One of the difficulties of blood-sugar studies is that the pain and fright of venous puncture may cause an elevation of the blood sugar. Experimentally, we have seen typical "diabetic blood-sugar curves" plotted from successive specimens from the heart of normal fasting rabbits. It is probable that the glycosuria of excited patients in neurologic and psychiatric clinics is of this type.

Clinically, emotional glycosuria is of especial importance in two groups of patients: nondiabetic subjects in whom the presence of glycosuria may lead to an improper diagnosis, and controlled diabetics in whom emotion may cause a return of glycosuria. Members of the first group are frequently found in out-patient clinics, especially among women, where intense emotion accompanies the thought of thorough examination, amid strange surroundings with the possibility of discovery of serious disease. It seems analogous to the temporary tachycardia or hypertension not rarely observed under similar conditions. Examinations of specimens collected by the patient at home or collected after he has become accustomed to the clinic will show a normal urine. The importance of postponing diagnosis in these patients is apparent. Among diabetic patients under satisfactory dietetic control a similar phenomenon is not unusual. For example, a mildly diabetic, middle-aged lawyer in our group tells me that he is always sugar-free except on days in which he pleads a case in court. A young, severely diabetic woman who had been sugar-free in the ward on a weighed diet had 12 gms. of sugar on the day she had a disagreement with the administrative department in regard to her bill; the matter was settled and she remained sugar-free without change in diet for over two weeks.

This type of glycosuria is believed to be due to "over-production" of glucose in the sense that, due to nervous stimulation of the liver, there is a rapid conversion of glycogen to glucose with the accumulation of the latter in the blood stream. Glycosuria is secondary to this elevation of blood sugar.

The pathogenesis of glycosuria in a number of other neurologic disturbances is probably the same as that of emotional glycosuria and is supposed to be analogous to that produced by the classical piqûre of the floor of the fourth ventricle by Claud Bernard. While the phenomenon has been observed in a large number of widely different disorders of the nervous system, it is of especial clinical importance in fracture of the base of the skull and intracranial hemorrhage, where the combination of unconsciousness and glycosuria may lead to the diagnosis of diabetic coma. Toxemia from various acute infections seems to behave in a similar manner in causing a temporary neurogenic glycosuria.

Another group of glycosurias with elevated blood sugar of some clinical importance are those associated with asphyxia from any cause. The mechan-

ism of this type is not understood, but it is usually assumed to be dependent on difficulty of oxidation. The two most familiar examples are found in carbon monoxide poisoning and after general anesthesia with either chloroform or ether.

By far the largest number of patients with glycosuria are suffering from disturbances of the glands of internal secretion. A number of these glands seem to importantly influence the metabolism of carbohydrate. Since glucose utilization and the basal metabolic rate have offered two simple quantitative measures of certain kinds of metabolic disturbances, these two methods of study have found wide application in the investigations of students of endocrinology. As is true of all studies in this field, interpretation of experimental results is difficult, much of the literature is speculative rather than scientific, and uncontrolled sources of error make conclusions dangerous. In few cases, however, we may safely apply the results of these investigations to the clinical interpretation of glycosuria.

That a disturbance of carbohydrate metabolism accompanies diseases of the thyroid gland in which there is a change in the quality or quantity of its internal secretion is well known. Glycosuria is a common feature of hyperthyroidism and of exophthalmic goiter, and in patients with these syndromes, hyperglycemia is the rule. The ingestion of glucose is usually followed by an abnormal elevation of the blood sugar with a delayed return to the fasting level, and sugar is found in the urine. Respiratory experiments demonstrate that there is no decrease in the patient's ability to utilize glucose. Glycosuria may be caused by thyroid feeding in man, and in animals it can be shown that thyroid gland or its extract causes a discharge of glycogen from the liver, and inhibits the deposition of glycogen newly derived from the food. The glycosuria of thyroid disease, then, is due to excessive mobilization of glucose rather than an inability to utilize it; this is in sharp contrast to the situation in diabetes mellitus.

Certain clinical characteristics of this type of glycosuria are worthy of mention. The sugar excretion is usually small in amount, and irregular. It does not run parallel to the carbohydrate in the diet, in contrast to true diabetic glycosuria in which all glucose above the patient's tolerance is excreted. High calory diets are followed by gains in weight and improvement in the general condition of the patient, even though there be a continuous glycosuria. Improvement in the thyroid disturbance will cause a disappearance of the glycosuria. Immediately following operation on patients with hyperthyroidism or exophthalmic goiter glycosuria is frequently noted. The characteristic signs and symptoms of thyroid disease are, of course, present, and those of diabetes are lacking. It must be remembered that loss of weight and polyphagia are characteristic of both. Following is an example of a patient with the glycosuria of hyperthyroidism who had been diagnosed diabetes mellitus.

CASE No. 3. An American, salesman, forty-six years of age, was referred to the Metabolic Clinic for treatment of diabetes mellitus. He dated his illness from an attack of whooping cough 18 months before. During his convalescence from this infection, he was refused life insurance because of glycosuria; two urinary examinations by other physi-

cians at this time were negative. At this same time he became very "nervous" and noticed that his hands "fumbled." Cardiac palpitation, shortness of breath and edema of the ankles followed a few months later, and his weight fell from 152 to 118 lbs. Examination showed a small goiter, cardiac enlargement with a systolic murmur at the base, pulse rate 110, fine tremor on extension of the fingers and a peculiar psychic state suggesting an anxiety neurosis. His urine contained sugar but was negative to ferric chloride. The blood sugar before breakfast the next morning was 0.14 per cent. Orthodiagram confirmed the cardiac enlargement. On a number of determinations his basal metabolic rate was between plus 27 per cent and plus 46 per cent. His blood pressure was 145 systolic and 65 diastolic, and there was a moderate lymphocytosis. During a week when he was on a diabetic diet, his urinary glucose for 24 hour periods varied from none to 10.7 gms. After the diagnosis of hyperthyroidism was reached, he was given a "house diet," and sugar was regularly absent from the urine. Usual medical measures combined with later ligation of the superior thyroid arteries has resulted in subjective cure, though the basal metabolic rate is still somewhat elevated. He has reported frequently for examination, and two years after admission has always been sugar-free.

To have attempted to control the glycosuria of this patient by diabetic diets would not only have subjected him to unnecessary discomfort but would have been contrary to the ordinary principles underlying the dietetic treatment of hyperthyroidism.

Patients with acromegaly or with pituitary gigantism frequently have glycosuria. These conditions are both believed to be caused by hypersecretion of the hypophysis, and there is evidence that the glycosuria is due to hypersecretion of the posterior lobe. It is not entirely improbable that the piqûre glycosuria is in some way related to the hypophysis. Pituitary glycosuria is clinically important only in that it may lead to a diagnosis of pituitary disease.

The secretion of the adrenal glands has recently become of clinical significance because it has been suggested as an antidote for insulin hypoglycemia. Adrenalin administered subcutaneously causes a discharge of glycogen from the liver into the blood, and in normal animals there may be a sufficient hyperglycemia to cause glycosuria. Its success as an antidote for insulin is not, however, uniform. In severe, undernourished diabetics, for example, the glycogen content of the liver is so small that only a negligible amount of glucose is added to the sugar in the blood. Several writers have pointed out recently that too much reliance should not be placed on this method of controlling insulin reactions.

Because of its frequency, its seriousness and its response to treatment, diabetes mellitus is by far the most important cause of glycosuria. Diabetes is one of the most common, if not the most common, serious, chronic disease in the United States, and while the expectancy of life of the uncontrolled diabetic is some three years, it is probable that if he is kept constantly sugar-free on a maintenance diet, he may live as long as his normal brother.

The term diabetes should be restricted to those cases of glycosuria in which the abnormality is in the utilization of glucose rather than in its mobilization or ingestion. During the recent past we have separated from the group that formerly was called diabetes the syndromes that have been described above, and it is possible that in the future other conditions may be distinguished. According to our present conception, all true diabetes mellitus

is due to functional disturbance of the Islands of Langerhans of the pancreas, even though the anatomic lesion may not be clearly demonstrable by pathologic methods. Putting aside the controversy concerning the mechanism whereby the pancreatic secretion is essential for the utilization of glucose, we are clearly safe in stating that without this secretion, glucose is not available to the body. It is not improbable that in the future we shall find that we are including among our diabetics a few patients whose defect is not pancreatic. Until these are recognized and methods of identifying them are elaborated, we shall have to continue to classify them as diabetic.

In the presence of the characteristic symptoms of glycosuria, hyperglycemia, polyuria, polydipsia, loss of weight and progressive weakness, the diagnosis is always clear. Similarly, we are usually safe in diagnosing diabetes when the characteristic complications are presented: gangrene, cataract, peripheral neuritis, pruritis vulvae, etc. Difficulty in diagnosis is met in three groups: patients with symptomless or nearly symptomless glycosuria; patients complaining of complications only; and patients in coma.

The symptomless diabetic is found during the course of life insurance or other routine examination. Understanding that diabetes is a serious and eventually fatal disease, he comes to his physician for advice. This is a situation in which the physician may do an enormous amount of harm. If the patient has one of the glycosurias described above, efforts at dietetic control will cause needless suffering to the patient, be ineffective in controlling and may actually aggravate his disease. On the other hand, to dismiss him casually, with the assurance that his glycosuria is negligible may throw him into the class of uncontrolled diabetes where he is certain to meet catastrophe. A careful and thoughtful diagnosis must be made.

It is a safe rule to regard all glycosuric patients as diabetic until they are clearly proved otherwise. Evidence obtained from a careful history may be important. If there has been diabetes in the immediate family, nondiabetic glycosuria should be diagnosed with unusual caution. The weight curve may be suggestive, especially if a large gain has been followed by a loss, or if there had been a definite fall from a previous obesity. Symptoms and signs of characteristic complications may be elicited only after careful questioning and thorough examination; important among these are peripheral neuritis, neuroretinitis, pruritis vulvae and chronic cholecystitis. Alimentary and emotional glycosuria can usually be eliminated from the diagnostic field by proper glucose tolerance tests. Untreated diabetics should have sugar constantly in the urine; intermittency of glycosuria—except in patients suffering from a complicatory infection—is evidence against diabetes. Finally a diabetic has a definite limit to his ability to utilize glucose; all that he absorbs above that level is excreted. Therefore, his glucose output parallels his glucose intake. It goes without saying that the presence of definite disease of organs of internal secretion mitigates against the diagnosis of diabetes.

Failure to diagnose the second group, in which the patient presents himself because of complications, is entirely a matter of negligence. This happens not only when the complication is entirely independent of the diabetes, but also when the complication should suggest diabetes. The only way in

which such an error can be prevented is by routine urinalyses and careful negative histories of all patients. We have under our care now a diabetic patient in whom a two-stage prostatectomy had been performed, with discovery of the diabetes when the wounds failed to heal after the second operation!

Still worse, however, are the patients suffering from well-known sequelæ of diabetes in whom the diagnosis of the underlying disease has been missed. A distressingly large number of patients come to us every year who have been treated locally for neuritis, neuroretinitis, gangrene, pruritis vulvae, boils, etc., with no urinalysis. The patient may have no interest in any systemic conditions, and even when he has symptoms of diabetes, will often volunteer no information. He has come for treatment of a local condition, and confines his description to that condition. Yet only control of the general disease will relieve the local symptoms. The following history of an indolent, spontaneous ulcer with failure of healing should have suggested diabetes, but it was not until the patient came to the hospital that the diagnosis was made.

CASE No. 4. A farmer, thirty-seven years old, was transferred October 2, 1922, to the Metabolic Clinic from the Department of Bone and Joint Surgery, which he had entered the day before with a painful and swollen foot. In July he had developed redness and pain about a callus on the right great toe, had incised it himself and expressed a small amount of pus. The toe became more painful, and he consulted a physician. Various local treatments were tried without success, and on September 15, the toe was amputated. The wound failed to heal and the anterior part of the foot became swollen and painful. He insisted that no urine specimen was examined during the four months of treatment. During this time he had noticed a very moderate polyuria and thirst, and had lost 7 lbs. in weight. Aside from arterial sclerosis and lost tendon reflexes, his general examination was negative. The great toe of the right foot had been removed with the first metatarsal bone, and the stump as well as the incision along the inside of the foot was open; pus could be expressed at several points. The foot, in the neighborhood of the wound was hot, reddened, swollen and tender. By x-ray, periostitis of the 2nd and 3rd metatarsals was easily demonstrated. The Department of Ophthalmology report of his optic fundus was: "Hemorrhagic retinitis with edema, hemorrhages both superficial and deep, with central degenerative changes. (Diabetic retinitis)." The patient was readily desugarized, and he tolerated without glycosuria a diet containing 2400 calories. His foot improved rapidly without surgical interference, and he was discharged January 19 with his foot healed.

The third group of glycosuric patients in whom diagnosis may offer some difficulties contains those who are in coma when first seen. In the emergency service of a hospital in the large city such cases are frequent, and having in mind diabetes as a possible cause of coma, the physician examines the urine. While the presence of sugar is strong evidence in favor of the diagnosis, it is not conclusive. It has been pointed out that fracture of the base of the skull is commonly accompanied by glycosuria, and Foster describes such a case in which a diagnosis of coma diabeticorum was reached in which autopsy revealed a small hemorrhage in the medulla. The following two cases are examples of the difficulty of diagnosis in some of these cases. In one an adenoma of the pituitary was found, while the other, a known diabetic, died in uremic coma.

CASE No. 5. A factory hand, sixty years of age, was sent to the hospital by his home physician for treatment of diabetes mellitus; in a letter sent with the patient it was

stated that he had been irrational during the past few weeks. When he arrived at the hospital he was in coma and little history was obtained. It was learned from a relative that he had used large quantities of morphine for the relief of headaches, that his vision had failed and that he had been for some time incontinent of urine and stool. On physical examination it was noted that he presented the appearance of dystrophia adiposa genitalis. His breathing was shallow, and not increased in rate. His pupils were contracted and did not react to light. The left knee jerk was exaggerated, the right sluggish, and the left leg seemed spastic. The blood sugar was 0.25 per cent, the urine contained sugar and gave a strong reaction with ferric chloride. At autopsy a large adenoma of the pituitary gland was demonstrated. While the clinical diagnosis was diabetic coma, it seemed, in view of the symptoms, neurologic signs and autopsy findings that the whole syndrome may have been pituitary.

CASE No. 6. A housewife, forty-four years of age, was entered in the Metabolism Ward with a diagnosis of toxic psychosis, with diabetes mellitus. She was completely disoriented, as to time, place and persons, and talked incessantly in a state of marked excitement. No history could be obtained. On examination, emaciation, dryness of the skin and absent knee and Achilles reflexes were noted. There was a high grade of peripheral arterial sclerosis, but the blood pressure was normal. The urine contained large amounts of glucose, "acetone bodies" and albumen, and in the sediment were many casts and pus cells. The blood sugar was 0.50 per cent. She was given large doses of insulin, and her blood sugar fell to a nearly normal level and her urine became sugar-free and almost ketone free. In spite of this her frenzy became worse, followed a few hours later by drowsiness and later coma. At this time the nonprotein nitrogen of her blood was found to be 75 mg. per 100 c.c. rising to 120 mg. and finally to 160 mg. She died in coma 96 hours after admission, with her urine sugar- and ketone-free. The death of this patient in coma with the high blood nitrogen, in spite of the fact that her diabetes had been controlled for two days, was sufficient evidence to convince us that the cause of death was uremia.

An occasional patient with glycosuria is encountered whom it is difficult or impossible to place in any clear-cut group. This may be because he combines two of the syndromes, or because there is no recognized entity which agrees with his disturbances. That the former coincidence might be expected to occur is obvious. Diabetes mellitus in particular is so common a malady that it would not be surprising to find it some times accompanied by hyperthyroidism, and acceptable cases of this combination have been reported. Likewise there is authority for the belief that there may be an important renal element in some cases of true diabetes. The other group, whose members appear at intervals in any large clinic, is even more difficult to understand. To these cases we are able to give no name other than "glycosuria of unknown origin" analogous to the obscure fevers and headaches of unknown origin. Citation of an example is more satisfactory than an attempt to describe the group. A number of similar cases might be described.

CASE No. 7. A salesman, twenty-four years of age, came to us for study after he had been refused life insurance because of glycosuria. He had no other symptoms, and had always been unusually healthy. During his college course, which he had finished 6 months before, he had been very athletic, and had achieved international fame on the track. He was in training for an important meeting during his stay in the hospital, and when his presence was not required at the hospital, he spent his time on the running track. His physical examination was negative, and except for the abnormalities in glucose metabolism, the laboratory examinations revealed nothing of interest. In the Out-Patient Department his urine was found to reduce Fehling's solution, but his blood sugar was

reported as 0.12 per cent. (Blood sugars were estimated by Benedict's modification of the Lewis-Benedict method.) He was sent into the ward for more careful study, his twenty-four-hour urine contained a large amount of glucose but the fasting blood sugar was only 0.10 per cent. He was given 1.75 gms. of glucose per kilogram of body weight, with the following results:

TIME	URINARY SUGAR	BLOOD SUGAR
Fasting	0	0.103 per cent
1 Hour	xxx	0.200 per cent
2 Hour	xxxx	0.130 per cent
3 Hour	0	0.100 per cent

This is not a normal reaction in that the blood sugar rose to too high a level, and there was glycosuria. On the other hand it was not a diabetic reaction because the peak of the blood-sugar curve was reached too early, the fall was too rapid and the glycosuria disappeared too soon. It was found that this reaction to his test meal was characteristic of his reactions to his ordinary meals. Before each meal, he was sugar-free, but a specimen collected two hours after he ate always showed a remarkable glycosuria. What seemed still more unusual was that the reaction was no different after a low calory diabetic meal with a small amount of available glucose from what it was after a "house tray," with or without sugar. Exercise had no influence on the phenomenon. Every precaution against malingering was taken, though there was no reason to believe that he was attempting to mislead us. We were able to give him no diagnosis other than "glycosuria of unknown origin."

A discussion of the significance of glycosuria cannot be limited to questions of diagnosis and prognosis. A few words must be said about its importance in the treatment of diabetes mellitus. In the days before insulin when we were entirely dependent upon dietetic control of the disease, careful students of the subjects were agreed that the prime requisite of the diet was that it keep the patient sugar-free. It was known that the patient utilized more sugar when his urine contained no glucose than he did during periods of glycosuria. Persistent glycosuria was recognized as an important cause of loss of tolerance, and there was even some evidence that the well-controlled diabetic might gain tolerance. There was general acceptance of the postulate that most of the complications could be avoided if the diabetic was maintained without glycosuria. Surgical procedures were, if possible, preceded by desugarization. In short, there was little dissension to the belief that the sugar-free diabetic was the safe diabetic, while the diabetic with glycosuria was in danger.

As insulin has become widely used in the treatment of diabetes, however, there has developed an unfortunate laxity in this matter. Because of the fact that a subjective improvement is obtained from liberal diets when this preparation is used, even though glycosuria continues, many diabetics are allowed to excrete sugar. Sometimes this glycosuria is permitted as a factor of safety against an overdose; more often it is the result of carelessness or negligence. As yet we have no reason for discarding the principles on which the treatment of diabetes had been based in the past. The sudden change from insistence on strict freedom from glycosuria to carelessness in this regard is neither reasonable nor consistent. There has, on the other hand, accumulated a rather large body of evidence that the diabetic who is only partly controlled by insulin is subject to the same accidents as is a milder

uncontrolled diabetic. Infectious complications are frequent, and what is more important, loss of tolerance has been observed. While sweeping conclusions may not be drawn from the limited data now at hand, the contrast between the two cases cited below is striking and suggestive. Both were young diabetics, originally of about the same degree of severity, who received similar doses of insulin over approximately the same period of time. The one who was careful to keep himself sugar-free maintained his tolerance; the one who permitted himself constant glycosuria lost most of his remaining ability to utilize glucose.

CASE No. 8. An instructor in the University, twenty-two years of age was admitted to the Metabolism ward in coma. His diabetes had appeared about 3 years before. He responded promptly to insulin therapy, and was eventually fed a liberal diet (3100 calories daily) with sufficient insulin to keep him sugar-free. While in the hospital he was fed a diet somewhat above his tolerance, without insulin, and excreted an average of 13.8 gm. glucose daily. He was an unusually intelligent and cooperative patient, who was able to vary his dose of insulin between 30 and 40 units daily so as to keep himself sugar-free nearly all the time. At the end of five months, after gaining 33 lbs. in weight, he returned at our request, was put on the original test diet without insulin, and excreted an average of 14.0 gms. glucose daily. His tolerance, as far as could be told, was exactly the same as it was five months before.

CASE No. 9. A civil engineer, twenty-eight years of age, entered the hospital extremely emaciated and weakened by diabetes of three years' duration. Between 30 and 40 units of insulin daily were required to keep him sugar-free on a diet allowing him 2950 calories daily. On a test diet, without insulin, he excreted an average of 16.8 gm. glucose daily. During the following five months he used 40 units of insulin daily, gained 30 lbs. in weight, regained his normal strength but had a constant glycosuria. At the end of five months, his glucose average daily excretion on his original test diet was 49 gm.

It will be noted that these two cases are quite similar with the exception of glycosuria. The age of the patients, the duration of the disease, the insulin dosage, the energy content of the diet and the gain in weight were nearly identical. One was strict in controlling his glycosuria; the other ignored it entirely. The former maintained his tolerance; the latter suffered a conspicuous loss. It is, of course, impossible to say that the second patient would not have lost tolerance had he remained sugar-free. However, the results of the two methods of treatment are so well in accord with our previous knowledge of the course of the disease, that they may be accepted as at least very suggestive. Until glycosuria can be shown to be harmless to the diabetic receiving insulin, it is not good therapy to permit it to continue.

Insistence on freedom from glycosuria greatly complicates the use of insulin in the treatment of diabetes. The variation in dose that may be required to keep a patient sugar-free is often extraordinarily large. This difficulty in keeping the balance between the insulin and the diet is a strong argument against the use of insulin in the case of anyone who can be fed a maintenance diet without the drug and without glycosuria.

SUMMARY

This discussion may be summarized as follows: Any glycosuria should be considered diabetic until clearly proved otherwise. On the other hand,

it must not be forgotten that there are a number of other syndromes in which glycosuria is a symptom. The more clear-cut of these clinical entities have been pointed out and some of the essential features of their diagnosis described. Emphasis has been placed on the importance of maintaining the diabetic in a sugar-free state, even if he is very mild or very severe, whether dietetic treatment alone be used, or insulin be used as an aid to dietetic treatment.

METHODS OF PRESERVATION OF SPECIMENS OF BLOOD INTENDED FOR THE DETERMINATION OF THE NONPROTEIN ORGANIC CONSTITUENTS*

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IT is frequently desirable to keep samples of blood intended for chemical analysis for relatively long periods until some suitable time or place becomes available for work. We are under the impression that most workers in this field believe that blood samples stored in a domestic refrigerator for 24 hours are suitable for the determination of all of the ordinary organic nonprotein constituents with the exception of blood sugar, but few of us would venture to recommend that these determinations be carried out on blood which has stood at room temperature for one or two days, as in the case of samples sent through the mails.

Some years ago Denis and Aldrich[1] suggested the use of commercial formalin, (containing 40 per cent formaldehyde) as a preservation for bloods in which it was desired to carry out the determination of blood sugar. This method has now been in use in this laboratory for three years and has given excellent results when the amount of preservative recommended in the original paper is used (1 drop to 10 c.c. of blood), if, however, an excessive quantity of formalin (2 to 6 drops) is added, the blood on standing becomes thick and viscous and is therefore difficult to measure in a pipette, although its blood-sugar content remains unchanged. While we still consider the formalin method of preservation to be excellent for the purpose for which it was originally devised, i.e., the preservation of samples of blood intended for blood-sugar determinations, it has the serious drawback that urea and nonprotein nitrogen cannot be determined in blood preserved in this way.

The loss of blood sugar which occurs when drawn blood is allowed to stand is a phenomenon of such practical importance from the analytical side that much work has been done in an attempt to find some preservative that will prevent glycolysis and several reagents have been suggested for this purpose, without reference to their effect as the other blood constituents. Perhaps the simplest method consists in laking the blood[2] either with water or with substances such as Saponine. In our experience laking (with distilled

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water) will prevent glycolysis for a few hours only in blood kept at room temperature, a fact which greatly limits the usefulness of this procedure.

Another method has been that suggested by Aibara[2] who found that sodium fluoride added to blood was efficient in preventing glycolysis, while Ambard[3] has advocated the use of a mixture of potassium dihydrogen phosphate and sodium fluoride. Mercuric cyanide[4] has recently been proposed as an efficient preservative of blood intended for the determination of urea by the xanthydrol method.

As it became desirable in connection with work now in progress, to make use of a preservative which would prevent change not only in the sugar but also in the other organic nonprotein constituents of the blood, we have carried on a series of experiments with various reagents in the hope of finding some substance which would act as a preservative at relatively high temperatures for all of the nonprotein constituents.

Our experiments have been carried out on dog's and on human blood. The general method of procedure has consisted in placing portions of oxalated blood (usually 5 or 10 c.c.) in 25 c.c. Erlenmeyer flasks to which the preservative was added in dry form. The flasks were then stoppered and allowed to stand at room temperature which in this climate is relatively constant during the season in which this work was done (28.5°-31.0° C.). At appropriate times these portions of blood were laked with distilled water, precipitated and analyzed according to the Folin-Wu[5] System of blood analysis, blood sugar and uric acid being determined by the revised technic.[6] The analytical figures are presented below.

The results may be summarized as follows:

Laking blood with seven times its volume of distilled water will invariably prevent the loss of blood sugar for 8 to 10 hours at 30° C., but after this time the blood-sugar content falls rapidly, while a rise in the nonprotein nitrogen is usually observed.

Mercuric cyanide, 1 mg. per 10 c.c. blood, was found to be unreliable for the preservation of blood sugar for periods as short as 24 hours and as it was therefore unsuitable for our purpose its preservative properties were not further investigated.

Sodium tetraborate (commercial borax) used in a concentration of 20 mg. per 10 c.c. of blood acted as an efficient preservative for blood sugar for 24 hours at 30° C., but was unreliable for longer periods, even used in considerably higher concentrations than indicated above.

Sodium fluosilicate was tried out because of the fact that it is much more soluble than is sodium fluoride and should theoretically exert a greater preservative action than does the latter salt as it contains about 60 per cent of fluorine, whereas sodium fluoride contains only 42 per cent. When added in a concentration of 100 mg. per 10 c.c. of blood this salt was found to exert an efficient preservative action for 24 hours at 30° C., it was also efficient for periods of 48 hours as a preservative of all the nonprotein constituent except nonprotein nitrogen, which fraction always showed an unmistakable increase at the end of this period. Blood to which sodium silico fluoride has been added is useless for the determination of the alkaline reserve as the addition of this salt produces abnormally low values.

Sodium fluoride has been recommended by Aibara[2] as being an excellent preservative for blood sugar when used in a concentration of 0.2 per cent, although he presents no experimental data on the subject. This substance appears, after numerous trials, to be the most efficient and convenient preservative for blood we have yet found; when used in a concentration of 60 mg. per 10 c.c. of blood we have found the concentrations of blood sugar, nonprotein nitrogen, urea, creatine and uric acid to be unchanged after the samples had been allowed to stand for 48 hours at 28.5° to 31° C. It is also without effect on the alkaline reserve of the serum as determined by the apparatus of Van Slyke.

Sodium fluoride when used as a preservative may be mixed with the potassium oxalate, or as it is itself an excellent anticoagulant it may be used without the addition of oxalate in the proportion of 60 mg. to 10 c.c. of blood, although as it is rather insoluble it is necessary to shake the sample rather more thoroughly than when oxalate is also added. We have not found the mixture of sodium fluoride and monopotassium dihydrogen phosphate recommended by Ambard to be in any way superior from the standpoint of its qualities as a preservative to sodium fluoride, and therefore prefer to use the pure salt.

TABLE I

EXPERIMENTS WITH SODIUM FLUOSILICATE, SODIUM FLUORIDE AND AMBARD'S FLUORIDE PHOSPHATE MIXTURE SHOWING THAT THE ADDITION OF THESE SUBSTANCES TO BLOOD IS WITHOUT EFFECT ON THE DETERMINATION OF THE NONPROTEIN CONSTITUENTS

	MNG. PER 100 C.C. BLOOD			
	OXALATED BLOOD, NO PRESERVATIVE	OXALATED BLOOD +120 MG. Na_2SiF_6 PER 10 C.C.	OXALATED BLOOD +120 MG. NaF PER 10 C.C.	OXALATED BLOOD +150 MG. NaF, KH_2PO_4 MIXTURE PER 10 C.C.
Nonprot. N.	30.6	30.6	30.6	30.5
Urea, N.	14.6	14.4	14.55	14.5
Uric Acid	2.2	2.3	2.2	2.2
Creatinine	1.4	1.3	1.4	1.4
Blood Sugar	109.3	109.3	109.2	109.2
Alkaline Reserve	44.9	11.8	44.9	13.5

Experiments showing the preservative action of Sodium Fluoride:

Experiment I. Dog's oxalated blood was analyzed immediately and after it had stood for 24 hours 28.5 to 31° C. with the addition of 15 mg. NaF per 10 c.c. blood.

	at once	after 24 hours
Nonprotein nitrogen	37.5	38.1
Urea nitrogen	14.2	14.2
Creatinine	2.1	2.1
Blood sugar	104.1	104.1

Experiment II. Human oxalated blood was analyzed immediately and after it had stood for 24 hours at 28.5 to 31° C. with the addition of 60 mg. NaF 100 c.c. of blood.

	at once	after 24 hours
Nonprotein nitrogen	46.8	46.8
Urea nitrogen	27.0	27.0
Uric acid	4.36	4.16
Creatinine	1.4	1.4
Blood sugar	114.2	114.2

Experiment III. Dog's blood (not oxalated) was analyzed immediately and after it had stood for 48 hours at 28.5 to 31° C. with the addition of 50 mg. NaF per 10 c.c. of blood.

	at once	after 48 hours
Nonprotein nitrogen	37.5	37.5
Urea nitrogen	18.75	18.72
Creatine	1.9	1.9
Blood sugar	208.3	208.4

Experiment IV. Human oxalated blood was analyzed immediately and after it had stood for 42 hours at a temperature of 28.5 to 31° C. with the addition of 60 mg. NaF per 10 c.c. of blood.

	at once	after 42 hours
Urea nitrogen	14.25	14.25
Uric acid	2.25	2.28
Creatine	1.3	1.3
Blood sugar	123.0	121.3

Experiment V. Human oxalated blood was analyzed immediately and after it had stood 68 and 93 hours at 28 to 31° C. with the addition of 200 mg. NaF per 10 c.c. of blood.

	at once	after 68 hours	after 93 hours
Nonprotein nitrogen	41.4	54.3	54.3
Urea nitrogen	19.9	19.5	19.7
Creatine	1.4	1.4	1.4
Blood sugar	166.6	167.1	166.6

From the experimental work described above it will be seen that blood preserved with sodium fluoride remains unchanged even at summer temperatures for as long as 48 hours, after longer periods (93 hours) no change is noted in the urea and blood sugar but the figures for nonprotein nitrogen are distinctly high.

Experiments showing the Preservative action of Ambard's Sodium Fluoride Acid Potassium Phosphate Mixture:

Experiment I. Human oxalated blood was analyzed immediately and after it has stood for 24 hours at 30° C. with the addition of 150 mg. of Ambard's mixture (1 part NaF+ 2 pts. KH_2PO_4) per 10 c.c. of blood.

	at once	after 24 hours
Nonprotein nitrogen	27.6	27.0
Urea nitrogen	13.1	13.3
Creatinine	1.39	1.4
Uric acid	2.0	2.2
Blood sugar	41.6	41.6

Experiment II. Procedure the same as in Experiment I, except that the storage period was extended to 48 hours.

	at once	after 48 hours
Nonprotein nitrogen	28.85	38.7
Urea nitrogen	14.2	14.2
Creatinine	1.5	1.5
Uric acid	2.25	2.24
Blood sugar	123.4	123.4

Experiment III. Procedure the same as in Experiments I and II, except that the observation period was extended to 116 hours.

	at once	after 116 hours
Nonprotein nitrogen	27.6	36.0
Urea nitrogen	13.1	12.9
Creatinine	1.39	1.39
Uric acid	2.0	2.0
Blood sugar	41.6	41.6

The above experiments indicate that while the fluoride phosphate mixture will preserve unchanged the nonprotein constituents of the blood for 24 hours it is not efficient as a preservative of the nonprotein nitrogen fraction for as long as 48 hours, and is in our opinion not as convenient or desirable as sodium fluoride.

Experiments showing the preservative action of sodium fluosilicate:

Experiment I. Human oxalated blood was analyzed immediately and after it had stood for 24 hours at a temperature of 29 to 31° C. with the addition of 60 mg. Na_2SiF_6 per 10 c.c. of blood.

	at once	after 24 hours
Nonprotein nitrogen	46.8	46.8
Urea nitrogen	27.0	27.1
Uric acids	4.3	4.3
Creatinine	1.4	1.4
Blood sugar	114.2	114.2

Experiment II. Procedure the same as in Experiment I, except that the storage period was extended to 42 hours and 100 mg. of sodium fluosilicate was added to 10 c.c. of blood.

	at once	after 42 hours
Nonprotein nitrogen	29.85	28.7
Urea nitrogen	14.25	14.25
Uric acid	2.25	2.25
Blood sugar	123.4	122.9

Experiments in which the attempt lengthens the time of efficient preservation by an increase in the amount of sodium fluosilicate used have been invariably unsuccessful so that we feel justified in making the assertion that this preservative cannot be relied on for periods of more than 24 hours for the nonprotein constituents other than nonprotein nitrogen and for 24 hours only for the latter fraction.

SUMMARY

Sodium fluoride when added to blood in a concentration of 60 mg. per 10 c.c. of blood, either alone or with potassium oxalate, acts as an efficient preservation for blood sugar, urea, nonprotein nitrogen, creatinine and uric acid for periods of 48 hours at a temperature of 28.5° to 31° C.; and for all of the above-mentioned constituents except nonprotein nitrogen for periods as long as 96 hours.

The mixture of sodium fluoride and potassium dihydrogen phosphate suggested by Ambard is distinctly inferior as a general preservation to sodium fluoride. Sodium fluoride will under the same conditions preserve blood for 48 hours for all the nonprotein constituents except nonprotein

nitrogen; for this latter fraction it is an efficient preservative for 24 hours only. The addition of sodium fluoride to blood does not interfere with the use of the serum for the determination of the alkaline reserve. Blood to which sodium fluosilicate or Ambard's mixture have been added cannot be used for this determination.

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THE CALCIUM CONTENT OF THE BLOOD IN PATHOLOGIC CONDITIONS*

By Irene Koechig, St. Louis, Mo.

DURING the past two years the writer has determined the amount of calcium in the blood in various pathologic conditions with a view of learning the degree of variations in the amount and whether abnormally low or high amounts would be found in any conditions other than tetany and rickets[1, 2] and uremia[3] in which low plasma calcium has been already observed.

Lyman's method of determining the calcium[4] was used, but all the determinations were made on citrated plasma, 0.25 c.c. of a saturated solution of sodium citrate being added to samples of from 10 to 25 c.c. of blood, as recommended by Halverson and Bergeim.[5] Control determinations were repeatedly made on solutions of known concentration, on beef blood and by running duplicate determinations by ashing. During the work it occurred to us that more or less calcium might be held in the protein precipitate formed by trichloracetic acid, especially when the proportion of plasma to acid is large (1:3). It appears, however, that the error is small. When the same plasma was precipitated in different volumes of acid and aliquots of each filtrate taken for analysis, the results varied irregularly as shown in Table I. The extent of these variations indicates the error of the method to be about 0.2 mg. calcium per 100 c.c. plasma.

Table I

The Effect of Varying the Proportions of Trichloracetic Acid (6.5% Solution) Used in Precipitating the Proteins of Plasma

Proportion of plasma to total vol.	Plasma I	Plasma II
1:4	10.5	9.85
1:8	10.7	9.90
1:12	10.55	10.05

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NORMAL VALUES FOR CALCIUM CONTENT OF PLASMA

Determinations on the plasma of eleven normal subjects gave results from 9.5 mg. to 11.0 mg. and averaging 10.3 gm. per 100 c.c. plasma. These values are in agreement with those of Halverson, Moler and Bergeim,[3] Kramer, Tisdall and Howland[2] and von Meysenbug.[6] We may therefore take 9.5 to 11.0 mg. calcium per 100 c.c. plasma as representing the limits of normal values and compare with these the results found in various pathologic conditions.

Results in Disease.

Our results on pathologic subjects are summarized in Table II.

TABLE II

CALCIUM CONTENT OF PLASMA IN PATHOLOGIC CONDITIONS

CLINICAL DIAGNOSIS	NO. OF CASES	CALCIUM PER 100 C.C. PLASMA		
		HIGHEST	LOWEST	AVERAGE
Syphilis	4	11.1	10.2	10.7
Arthritis chr. inf.	2	11.3	9.8	10.5
Diabetes	3	10.8	9.9	10.3
Diabetes renal	1			9.5
Septicemia	2	10.9	9.8	10.3
Septicemia (acute nephritis)	1			9.1
Septicemia—after three days calcium lactate.				9.6
Anemia, Pernicious	3	10.5	9.5	9.9
Myelogenous Leukemia	3	10.7	9.8	10.3
Staph. Dermatitis	1			10.7
Typhoid Fever	2	10.7	9.8	10.3
Nephritis (severe)	1			9.1
Uremia	1			8.1
Raynaud's disease	1			9.9
Purpura Hemorrhagica	1			10.4
Furunculosis	1			10.8
Pleurisy suppurative	1			10.4
Constipation chr.	2	10.4	9.9	10.1
Dysentery colitis chr.	1			9.3
Dystrophy progressive muscular	1			10.1
Tetany	1			8.1
Epilepsy (during petit mal)	1			10.3
Cocaine poisoning (convulsions)	1			10.6
Digitalis Poisoning	1			10.2
Pellagra	1			8.8
Osteomalacia	1			7.4
Achondroplasia	1			9.7
Multiple Fractures	1			10.0
Fractured Femur	1			10.1
Fractured Pelvis (path. fracture destructive cond. of bone)	1			11.8
Paget's Disease	2	12.1	11.8	11.9
Sclerosis (disseminated)	1			10.7
Pneumonia	4	10.4	9.9	10.2
Pneumonia (acute hemorrhagic glomerular neph.)	1			8.6
Influenza	3	11.2	9.9	10.6

The chief point to be noticed is the great constancy of these figures. During the past three years several papers have appeared that seemed to show a very great variation in values of the calcium content of the plasma and serum both in pathologic conditions and in normal subjects. This is entirely at variance with our experiences and for this reason we have wished

to record our results. A decrease in calcium was observed in a case of nephritis with uremia (8.6) while the milder renal, cardiorenal, and cardiac cases showed little decrease. A case of dysentery and chronic colitis showed a slight decrease (9.3 mg.) in the calcium content of the plasma possibly due to an increased excretion of calcium into the large intestine. A single case each of pellagra (8.8 mg.) and of osteomalacia (7.4 mg.) showed a distinct decrease in plasma calcium as did also a case of adult tetany (8.1 mg.). Only three cases showed an increase of the calcium content of the plasma, two of these being Paget's disease (11.8 and 12.1 mg.) and the third a pathologic fracture in which the x-ray findings showed a destructive condition of the bone (11.8 mg.).

BLOOD CALCIUM IN CARCINOMA

In his studies on malignant growths, Beebe[7] found that old degenerated carcinoma and sarcoma contained much more calcium than did the more active tumors. This suggested that in such cases there might be a disturbed calcium metabolism so a number of these cases were investigated. It was soon found that a part showed a marked decrease in the plasma calcium and that these cases were markedly jaundiced, while in those cases in which there was no jaundice the calcium values lay within the normal limits. It therefore seemed possible that the lowered values found in the carcinoma cases might not primarily be due to the carcinoma but rather to the jaundiced condition of the blood, which would be in accord with the work of Halverson who found a similar reduction of calcium in hematogenous jaundice.[8]

TABLE III

COMPARISON OF CARCINOMA CASES JAUNDICED AND NOT JAUNDICED

NO JAUNDICE		JAUNDICE	
Carcinoma prostate	11.1	Carcinoma	10.7
Carcinoma prostate	9.8	Carcinoma	8.9
Carcinoma prostate	11.0	Carcinoma	8.1
Carcinoma esophagus	10.3	Carcinoma	7.8
Carcinoma neck glands	9.6	Carcinoma pancreas	9.0
Carcinoma stomach	9.9	Carcinoma pancreas	8.7
Carcinoma trachea	10.8	Carcinoma	8.8
Sarcoma of back	9.9	Gall stones	9.3
		Cirrhosis of liver	9.1
		Cirrhosis of liver	9.2

In making the determinations on the blood of jaundiced cases it was observed that the protein precipitate obtained by treating the plasma with trichloracetic acid was markedly yellow in color changing to green upon standing. This was due to a retention of the bile pigments in the precipitates, the filtrates being water clear. Such behavior is characteristic of blood which contains an abnormally large amount of bile pigment and has been found useful as a test for this substance. A note on the detection of bile pigments in blood, urine and gastric contents will appear shortly. The presence of these pigments in the precipitate suggested the idea that the low calcium values found might be due to a retention of a part of the calcium as the insoluble salt of the bile pigment. To investigate this a number of

precipitates both from jaundiced and from normal bloods were thoroughly washed with trichloracetic acid to remove the blood filtrate and were ashed and the calcium thereof determined by the method employed above with the results shown in Table IV.

TABLE IV

COMPARISON OF CALCIUM CONTENT OF PROTEIN PRECIPITATE OF NORMAL AND OF JAUNDICED PLASMA

Ca PER 100 C.C. PLASMA MG.	Ca IN PRECIPITATE FROM 100 C.C. PLASMA MG.	TOTAL
Jaundiced		
9.3	1.7	11.0
8.1	1.27	9.37
7.8	1.91	9.71
9.0	1.70	10.70
8.7	1.17	9.87
Normal		
10.2	0.54	10.74
9.5	0.65	10.18
10.8	0.27	11.07
11.1	0.21	11.31
10.6	0.65	11.25

Thus the protein precipitates from the jaundiced plasma show a much larger amount of calcium than the precipitates of normal plasma; and if these values be added to the amounts found in the filtrates the totals approximate normal values. It would thus seem that the low results are only apparent and are due to the precipitation of some of the calcium, perhaps as a salt of the bile pigment. In jaundiced bloods an ashing method should be employed.

BLOOD CALCIUM IN TETANY

During these investigations there was under observation for a number of months an adult epileptic with tetany associated with a sinus infection and with hyperacidity and dilation of the stomach. Previous to the hospital admission and during most of this period alkali therapy was employed and frequent gastric lavage was used. No definite relation between this and the convulsions could be established. These tetanic convulsions were differentiated from the epileptic fits by the presence of tonic spasms of the upper extremities resulting in the typical "obstetrical hand" attitude, by contraction of the facial muscles, by the retaining of consciousness, and by spasms of the abdominal muscles.

A series of calcium determinations on the plasma extending over a period of three months is shown in Chart I. The values found on different days varied from 6.4 to 10.9 mg. per 100 c.c. plasma, those obtained in periods of tetany being 8.2, 6.4 (plasma bicarbonate 58.4 vol. per cent), 8.6, 9.0, 8.7 and 9.5. This accords with the findings of Howland and Marriott[1] in infantile tetany and Barach and Murray[8] in tetany in a case of sprue in showing a lowering of the calcium content of the plasma, although with the exception of one figure (6.4) these reductions are slight and do not approximate the low values found in infantile tetany. Values of 8.1 and 8.7 were found in the absence of tetany while convulsions occurred with 9.5 mg. calcium; but such results are exceptional. These findings show a small fall in the con-

centration of calcium in the serum during active tetany but no definite level at which the symptoms begin. From this it would seem that there are factors other than the concentration of calcium in the plasma concerned in the production of adult tetany.

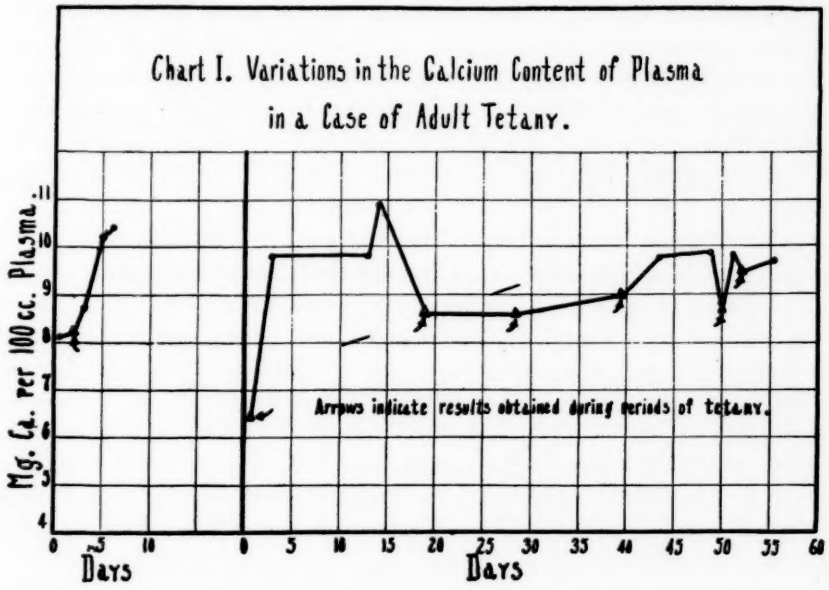
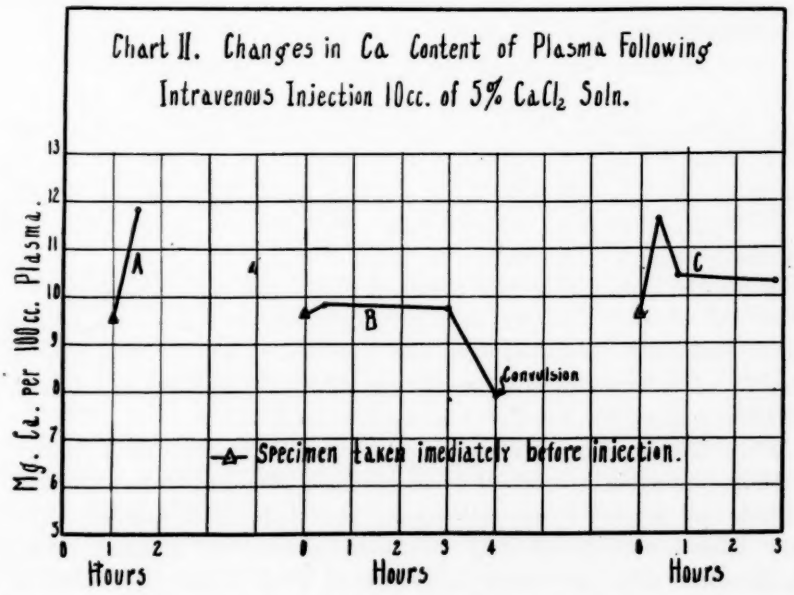


Chart I. Variations in the Calcium Content of Plasma in a Case of Adult Tetany.
Mg. Ca. per 100 cc. Plasma.
Arrows indicate results obtained during periods of tetany.
Days
Days



In none of the other cases showing an abnormally low calcium was a nervous hyperexcitability shown and, on the other hand, determinations on the plasma of epileptics taken immediately after convulsions and on cases of neurasthenia and psychasthenia showed normal values.

As a therapeutic measure, 10 c.c. of a 5 per cent calcium chloride solution was injected intravenously in this case and it was thought to be of

interest to follow the changes of the concentration of calcium in the plasma. The results of three such administrations are shown in Chart II. Unfortunately none of the initial specimens showed abnormally low values. In curve A, the calcium rose from 9.5 to 11.8 mg. calcium per 100 c.c. plasma in a half hour, but no subsequent specimens were obtainable. The patient felt much improved and no cramping occurred for three days. In curve B half an hour after the administration, the calcium was found to be 9.8, a value but slightly higher than the original 9.6. A few hours later it fell to 7.9 and a severe attack of tetany developed. In curve C the calcium rose from 9.6 to 11.6 and at the end of one hour had returned to 10.4 at which level it remained for the subsequent three hours. No tetany occurred for several days.

In none of these curves is an abnormally high concentration of serum calcium maintained which shows the difficulty with which anything more than transitory increases above the normal level are obtained. This has been shown by Clark[9] who found similar results with hypodermic injections of calcium hydroxide and of calcium chloride in rabbits. In cases of infantile tetany Brown[10] was unable to maintain even normal values by means of intravenous injections of calcium lactate but found temporary increases accompanied by the absence of the mechanical and electrical signs of tetany. In curve B it would seem that some condition existed which caused so rapid a removal of calcium that in the first half hour the amount had fallen practically to the original value, and after that continued to fall. Whether the marked fall and the tetany, following calcium injection was a coincidence we are unable to say.

SUMMARY

1. In various pathologic conditions the calcium content of plasma does not differ from the normal values, which appear to lie between 9.5 and 11.0 mg. Ca. per 100 c.c. plasma. Low results have been found in nephritis, colitis, pellagra, jaundice, osteomalacia, and tetany; and high results in Paget's disease and a pathologic fracture.

2. In plasma containing bile pigments low values for calcium are found. These low values are, however, *only apparent* and are due to a precipitation of calcium, probably combined with bile pigment in the protein precipitation. The amount of calcium in the precipitate added to the amount found in the blood filtrate brings the total within normal limits.

3. After intravenous injections of calcium chloride in a patient with adult tetany, the calcium content of the plasma rose and then returned to normal within one hour. It would appear therefore that calcium injection is not very effective in producing and maintaining an increased amount of plasma calcium. The low content associated with tetany is doubtless due to factors other than a mere insufficient supply.

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THE PHARMACOLOGY OF BENZYL ALCOHOL AND ITS ESTERS

III. Some Observations on the Effect of Benzyl-Benzoate upon Arterial Hypertension in Man*

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BENZYL-BENZOATE and benzyl-acetate were introduced into medicine as possessing blood pressure reducing qualities, by Macht[1] in 1918. Since their introduction a number of additional benzyl compounds[2] have been advocated as possessing similar properties. In the past four years these substances have been alluringly advertised by a large number of commercial houses and copiously prescribed by clinicians for a number of ills, among them being arterial hypertension. No one has yet made a careful clinical study to determine whether or not these substances really possess vasodilator or blood pressure lowering properties when taken by mouth in man.

In 1918 Macht[1] noted that benzyl-benzoate and benzyl-acetate produced a fall in arterial blood pressure in dogs and rabbits when injected subcutaneously, intramuscularly, intraperitoneally or intravenously. He recommended their use in human beings to relax contracted smooth muscle in a host of conditions. Two years later he[3] published a resumé of twelve cases in which benzyl-benzoate administered by mouth lowered the systolic pressure in amounts varying from 16 to 75 mm., and the diastolic from 10 to 60 mm. of mercury. He found it of value in all cases of hypertension, but notably so in idiopathic or essential hypertension. In respect to his belief in its efficacy he says: "I have found that after administering to a patient full doses of benzyl-benzoate and obtaining a desirable therapeutic effect, the reduced pressure could be maintained by keeping a patient on very small doses of the drug, sometimes no more than five minims of the twenty per cent solution." He noted also an appreciable vasodilator effect in some cases within thirty minutes after administration. Usually the effect did not appear as early as with the nitrites and when it did occur was of briefer duration than that observed after erythrol tetranitrate administration. This author, however, gives no description of how the blood pressure readings

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were made, nor does he mention whether the patient had been resting or was on a special diet at the time of medication.

In a series of articles which appeared recently, Nielsen and Higgins[2] confirmed Macht's findings in dogs. Laubry and Mougeot[4] likewise believe that benzyl-benzoate is of the utmost value in lowering the blood pressure. They administered it by mouth in the treatment of arterial hypertension in 20-drop doses three times a day. Although they cite no cases and give no histories of cases, nevertheless they note that benzyl-benzoate has an immediate and a prolonged action, the latter condition being observed only after repeated doses for several days. They write that 20 drops of benzyl-benzoate caused a fall in blood pressure of 20 to 30 mm. of mercury within the first half hour after administration, and that after repeated doses over a period of several days a prolonged fall was observed which lasted for some weeks after withdrawal of the drug.

Recently with neither clinical nor experimental evidence an article appeared in the literature by Cumston[5] in which he claims to have obtained beneficial effects after frequent administrations of benzyl-benzoate. He writes, "Benzyl-benzoate is given in doses of twenty drops in a five per cent, alcoholic solution nine times in twenty-four hours, but larger doses may be exhibited without any untoward effects. The hypotensive action of this drug is manifest. When injected into dogs a marked vasodilatation results in the domain of the splanchnic nerves, resulting in a lowering of the blood pressure. The drug may be given hypodermically in an oily solution containing from two to four drops in each cubic centimeter of olive oil."

On the other hand in 1920 Musser[6] found benzyl-benzoate to have no effect upon arterial hypertension in six cases in which it had been given for some weeks. A similar lack of results from benzyl-benzoate was cited by Allen and Sherrill[7] as they say, "Benzyl-benzoate has been tried only in severe cases and has never given the slightest benefit."

During the year 1920 Mason and Pieck[8] published a report on a series of experiments performed by them in which they found that benzyl-benzoate injected intravenously in dogs lowered blood pressure, but that this lowering of blood pressure was due, not to its action upon the blood vessels, but upon the heart. Recently this subject has been studied quite thoroughly by one of us[9] on dogs, cats and rabbits. It was noted that benzyl-benzoate, benzyl-acetate and benzyl alcohol did not lower blood pressure in dogs when given by stomach tube even in amounts 210 times the customary clinical dose. When given intravenously these substances increased blood pressure in 8 per cent of the experiments and decreased it in 90 per cent. The heart was the first organ to be affected. Here the benzyl compounds in small doses were found to decrease its volume whereas large doses caused dilatation of the organ.

Because of this marked variation in effect upon blood pressure in experimental animals and divergence in clinical observation among the various authors, this investigation was undertaken to determine more accurately if benzyl-benzoate has an effect upon clinical cases with arterial hypertension.

METHOD

In all instances the patients selected for this investigation had systolic blood pressures over 175 mm. of mercury at the beginning of observation. No gross cardiac lesions were found. With but one exception the blood of these patients gave a negative Wassermann reaction.

The blood pressure was taken with either a Tycos sphygmomanometer or a mercury manometer. The Tycos manometer was tested each day it was used against a mercury manometer to determine its accuracy. The maximum and minimum pressures were determined by the Korotkoff sounds[10] with a stethoscope. The fourth sound was taken as the minimum pressure.

The patients were placed in the recumbent position in a quiet room and blood pressure readings taken within two minutes after the patients had lain down and thereafter at ten minute intervals for thirty or more minutes. In all the observations the subjects were requested to lie as quietly as possible and not even to speak to the observer. As nearly as possible the blood pressure readings were taken the same hour each day. These were recorded for several days until the control blood pressure level was established before the drug was prescribed. In the early experiments readings were taken every day, but later were taken every second or third day. As nearly as possible the patients were kept on their regular diet, but in a few cases the coming of Lent necessitated change in diet.

Hynson, Wescott and Dunning's 20 per cent alcoholic preparation of benzyl-benzoate was used in 25- and 30-drop doses four times a day over a period of four to eighteen days.

In a number of cases after several control readings of blood pressure were taken, 25 drops of benzyl-benzoate were given and the blood pressure taken every fifteen minutes for two hours.

RESULTS

The value of rest in arterial hypertension.—The question of the value of rest in the treatment of hypertension cases is discussed by a number of investigators, Musser,[6] Mosenthal[11] and O'Hare[12] claiming that it is of value, and Allen and Sherrill[7] and Moschowitz[13] belittling its importance. A marked variation in blood pressure when taken at different times of the day was observed by Strouse and Kelman.[14] Upon studying sixteen cases over a period of a month or more, except in one case which proved to be a severe case of nephritis (see Table I and curves), we observed that rest uniformly caused a reduction in both the maximal and the minimal blood pressure readings (confirmation of O'Hare).[12] This reduction is observed only after the novelty of having the blood pressure taken frequently has worn off. It is necessary that there be both mental and physical rest for this reduction to take place. In Table I it will be seen that the blood pressures remain high even after 30 minutes of rest during the first few days. This we believe to be largely of psychic origin. The procedure was entirely new to the patients. They did not understand the reason for being put to bed and having the blood pressure taken every ten minutes. They were nervous

and restless. That excitement can cause an increase in blood pressure was well illustrated by O'Hare.[12] We, like O'Hare, found that exercise increases blood pressure in hypertension cases. We found that contracting the muscles of one arm while the blood pressure was being taken on the other would frequently raise the maximum pressure as much as 15 mm. of mercury.

The greatest reduction in blood pressure under temporary rest is observed in Case V in which the systolic blood pressures fell as much as 20 to 66 mm. and the diastolic pressures from 4 to 24 mm. of mercury. This change can also be noted in Figs. 1, 2, 3 and 4. Unless this factor was taken into consideration by Macht[3] and by Laubry and Mougeot,[4] their reductions in blood pressure by benzyl-benzoate could readily be explained by temporary rest alone.

Does benzyl-benzoate reduce blood pressure when taken by mouth?—Benzyl-benzoate as far as we could determine from sixteen unselected hypertension

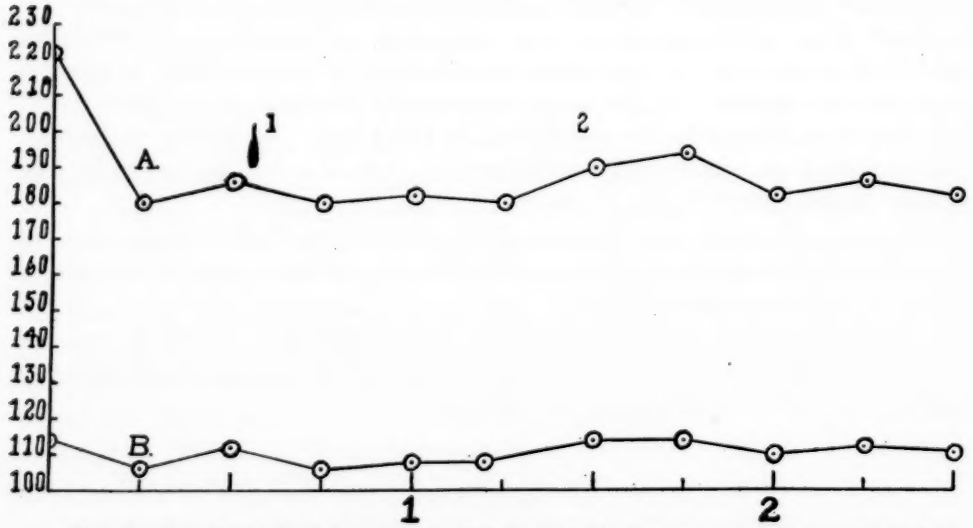

Fig. 1.—Record obtained from patient VII (see Table I). Upper curve, maximal blood pressure; lower curve, minimal blood pressure. Blood pressure in mm. of mercury plotted on the ordinates and time in hours on the abscissae. (*1*) Patient given 26 drops of 20 per cent alcoholic solution of benzyl-benzoate in 100 ml. water. (*2*) Observers were talking in the room but not to the patient.

cases, carefully controlled, had no immediate or remote effect upon either the maximum or minimum blood pressures.

Immediate Effect.—The lack of immediate effect (within 2 hours of administration) is well illustrated in Fig. 1. In this case 26 drops of a 20 per cent alcoholic solution of benzyl-benzoate were given by mouth at (1), after a normal had been established. Blood pressure readings were taken thereafter at fifteen minute intervals for two hours with no change. At (2) the observers talked in the room with each other, but not to the patient, with the resultant increase in pressure, as seen in the figure. In the cases thus studied, in not a single instance did benzyl-benzoate influence the arterial blood pressure. We found as did O'Hare[12] that nitroglycerin frequently produced either no change in blood pressure or a slight rise followed by a

slight fall. In one of our cases the heart was markedly accelerated and the systolic pressure was increased 12 mm. of mercury.

Effect of Repeated Doses.—The failure of repeated doses of benzyl-benzoate to lower blood pressure is well shown in Figs. 2, 3 and 4 and in all the cases except Case II in Table I. In patient XI, whose blood pressure is represented in Fig. 2, benzyl-benzoate treatment was started with 30 drops four times a day at (1). This was continued for 8 days, after which it was discontinued at (3). In this patient the medication certainly did not decrease the blood pressure; if it had an effect it was that of increasing it. Patient IX, whose blood pressure record is shown in Fig. 3, received 25 drops of benzyl-benzoate four times a day for the first two days at (1). This was increased on the third day to 30 drops four times a day. The patient took the drug for five days without showing any change in blood pressure. On the eighth day the blood pressure had fallen 20 mm. of mercury, but the patient was ill with a severe rhinitis and pharyngitis which continued for

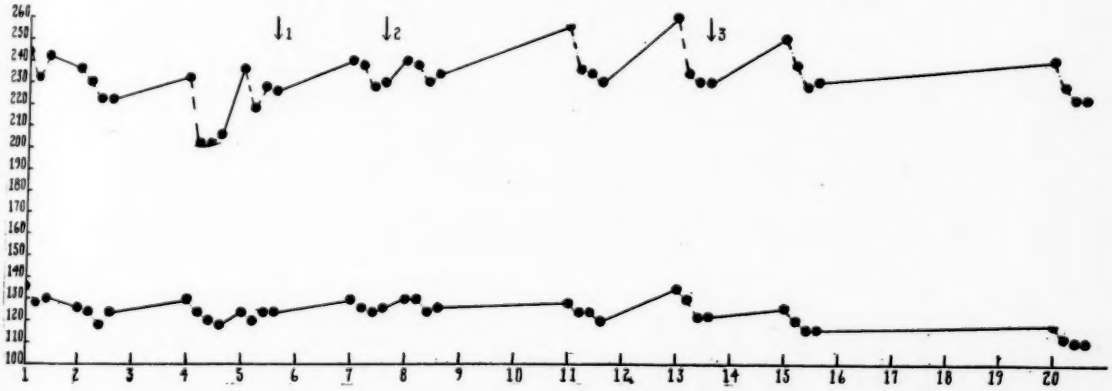

Fig. 2.—Patient II. In this and the following two figures the upper curve is the systolic blood pressure, the lower curve the diastolic blood pressure in mm. of mercury. Blood pressure in mm. of mercury plotted on the ordinates and time in days on the abscissae. Points connected by heavy lines are intervals of days; those connected by dotted lines are intervals of 10 minutes. (*1*) Patient given 30 drops four times a day. (*2*) Extra systoles present. (*3*) Drug discontinued.

some time. In all, the patient took the drug for eighteen days with no conclusive evidence that it was doing her good. The medication was withdrawn and the blood pressure continued the same for fourteen days thereafter, after which the patient failed to return to the dispensary. Fig. 4 was plotted from the data obtained from patient XII. In this instance as in the former case the patient was started with 25 drops of benzyl-benzoate four times a day at (1). At (2) it was increased to 30 drops four times a day. This was continued for thirteen days with no change in blood pressure. When the patient came into the dispensary on the thirteenth day of medication he was hiccoughing every 5 to 10 seconds. He stated that this condition had started two days before. Shortly thereafter the patient developed a severe bronchial infection which later cleared up, but the blood pressure remained 20 mm. lower than observed under benzyl-benzoate treatment. That acute infections can lower blood pressure is stated by Ives.[15]

TABLE I

This table gives a summary of important laboratory findings, blood pressure readings and drug therapy. The blood pressure readings taken on the day that benzyl-benzoate was prescribed were recorded before the patient received the drug, and those recorded on the day that benzyl-benzoate was discontinued were the last taken before the patient stopped it. Where found in table, T = trace; FT = faint trace; VFT = very faint trace; D = day; N = night; plus = positive; 0 = negative; H = hyaline; SG = small granular and LG = large granular. To conserve space the specific gravity of the urine is recorded (in parentheses) under casts and the blood uric acid (U. in the table) and creatinine (C. in the table) findings in mg. under N.P.N.

All cases gave a negative blood Wassermann reaction except Case XI. After mercury and potassium iodide administration this patient's blood gave a +++ Wassermann with cholesterin antigen. A previous blood Wassermann taken 2-1-22 was negative.

CASE NO.	LABORATORY FINDINGS			BENZYL-BENZOATE ADMINISTERED BY MOUTH	DATE 1923	BLOOD PRESSURE IN MM. MERCURY WITH AND WITHOUT REST				DIAGNOSIS AND REMARKS
	BLOOD N.P.N.	URINE P.S.P. %	URINE ALBUMIN	URINE CASTS			0 REST	10 MIN. REST	20 MIN. REST	30 MIN. REST	40 MIN. REST	
I			FT	FG		1-31	230-129	229-129	231-128			Chronic interstitial nephritis
Female				SG		2-1	236-126	229-125	228-126			Arteriosclerosis
white				(1.022)	25 m. t.i.d.	2-2	222-129	198-120	204-120	204-120		2-19 Acute bronchitis and rhinitis
age 58					25 m. q.i.d.	2-3	219-118	210-115	200-114	206-120		
					"	2-5	214-124	204-120	200-120	202-120		
	28.1				Discontinued	2-8	230-120	212-124	212-120			
						2-10	198-114	204-118	200-114	200-114		
						2-19	204-114	198-116	190-114	188-112		
		34				3-3	184-110	170-106	174-108	174-108		
II						2-1	184-98	184-98	182-98			2-4 Patient was nauseated, vomited, went to bed. 2-5 still very ill, sent home and told to remain in bed. Acute pharyngitis and bronchitis. 2-16 pneumonia
Female					25 m. t.i.d.	2-2	194-100	176-98	174-102	174-100		
white			Plus		25 m. t.i.d.	2-3	180-104	170-100	168-100	168-100		
age 41			3 plus	(1.020)	15 m. t.i.d.	2-5	160-100	154-94	156-96			
					20 m. q.i.d.	2-6	136-82	136-82	134-80			
					"	2-7	164-96	154-94	154-96			
	31.75				"	2-8	156-90	136-84	136-84			
					"	2-9	140-86	138-80	128-80	126-80		
					"	2-10	170-96	142-96	140-90			
					"	2-12	168-96	156-94	152-92	148-92		
					Discontinued	2-13	160-96	158-96	154-92	154-92		
					25 m. q.i.d.	2-15	160-94	156-92	154-94			
					"	2-16	168-98	154-96	152-94	152-94		

TABLE I—CONT'D

CASE NO.	LABORATORY FINDINGS				BENZYL-BENZOATE ADMINISTERED BY MOUTH	DATE 1923	BLOOD PRESSURE IN MM. MERCURY WITH AND WITHOUT REST					DIAGNOSIS AND REMARKS
	BLOOD N.P.N.	URINE P S P. %	URINE ALBUMIN	URINE CASTS			0 REST	10 MIN. REST	20 MIN. REST	30 MIN. REST	40 MIN. REST	
III Female white age 48			FT	(1.015)		2-1	204-116	194-116	192-116			Chronic interstitial nephritis
						2-2	226-118	194-116	188-114	188-110		
						2-3	178-110	172-110	168-110	174-114		
						2-5	170-114	166-114	164-110	154-108	154-108	
					25 m. t.i.d.	2-6	184-114	174-110	172-110	174-108		
					30 m. q.i.d.	2-7	170-112	172-108	176-108	174-108		
					"	2-8	188-114	175-106	172-104	172-104		
					"	2-9	180-110	178-110	162-110	158-110	158-108	
	25.6				Discontinued	2-12	188-108	172-108	170-112			
						2-13	194-110	174-110	162-108	160-106		
						2-21	170-102	154-94	158-96	148-90		
		39				2-28	200-118	170-110	158-108	160-110		
						3-7	188-114	164-108	170-110	182-112		
IV Female white age 62	34.3		plus	LG		2-1	184-110	182-108	180-108			Arteriosclerosis. Chronic bronchitis. Chronic interstitial nephritis 2-5 Nauseated and patient reduced dose of Benzyl-benzoate to 10 drops three times a day. 2-8 Acute bronchitis rhinitis and pharyngitis
						2-2	180-100	174-112	164-100	166-100		
					20 m. t.i.d.	2-3	172-104	164-100	164-102	162-102		
					15 m. q.i.d.	2-5	176-104	166-102	162-102	162-102		
					"	2-6	160-110	156-104	142-102	148-108		
			T	LG	20 m. t.i.d.	2-7	174-108	172-108	172-110	182-110		
					Discontinued	2-8	182-114	176-112	174-110			
						2-12	180-110	170-108	168-104			
						2-16	170-114	170-110	168-108			
						2-27	158-96	148-94	150-94	148-92		

TABLE I—CONT'D

CASE NO.	LABORATORY FINDINGS				BENZYL-BENZOATE ADMINISTERED BY MOUTH	DATE 1923	BLOOD PRESSURE IN MM. MERCURY WITH AND WITHOUT REST					DIAGNOSIS AND REMARKS
	BLOOD N.P.N.	URINE P.S.P. %	URINE ALBUMIN	URINE CASTS			0 REST	10 MIN. REST	20 MIN. REST	30 MIN. REST	40 MIN. REST	
V		43				2-2	246-122	224-116	218-116	220-114	218-112	Chronic interstitial
Male		42				2-3	240-122	224-118	226-120	220-118	220-120	Nephritis
white						2-5	260-126	236-126	214-112	204-118	198-116	Arteriosclerosis
age 57							160					Hemiplegia left
					25 m. t.i.d.	2-6	242-140	208-126	206-120	194-120	194-116	2-5; 2-7; 2-8 Pulsus
					30 m. q.i.d.	2-7	270 230-136	230-125	232-122	204-116	204-116	alternans present
	28.8		T	LG SG	Discontinued	2-8	242 260-136	206-130	204-122	200-120	202-120	
		43		(1.021)		2-10	228-126	200-116	200-116	200-116		
						2-14	242-126	228-112	204-110	200-106	192-102	
					30 m. q.i.d.	2-20	270-130	216-110	202-108	208-112		
					Discontinued	2-23	260-126	220-116	206-110	206-108		
VI						2-9	234-120					Hemiplegia right
Female			VFT	0		2-13	250-126	226-120	216-120	218-120		Arteriosclerosis
white					20 m. q.i.d.	2-14	258-138	244-136	236-126	238-128		2-14 patient suffering from severe cold
age 62	30.3				30 m. q.i.d.	2-15	262-140	244-132	240-126	238-128		
					"	2-16	250-126	220-116	218-116	218-118		2-20 patient developed pneumonia
					"	2-17	240-118	208-116	210-116	220-124		
					"	2-19	242-126	226-118	222-118	222-118		
VII			plus			2-15	220-138	208-132	198-128	192-129	192-126	Hemiplegia, old, right
Female						2-16	190-126	164-114	160-116	164-116		Observed Lent
white						2-17	180-120	168-116	162-114	164-116		
age 42	27		0	0	25 m. q.i.d.	2-19	190-118	170-116	160-114	158-114		
				(1.018)	"	2-20	180-112	166-110	158-108	164-112		
					30 m. q.i.d.	2-23	184-120	162-116	164-116	164-116		
		60			"	2-26	178-118	160-112	160-112	162-114		
					Discontinued	3-3	206-130	170-122	168-122	166-120		
						3-6	174-114	160-110	162-110	156-110	156-110	
						3-13	188-122	170-118	170-120	166-118		
						4-2	192-118	162-117	156-114	160-114		
						4-10	170-110	158-108	158-108			

TABLE I—CONT'D

CASE NO.	LABORATORY FINDINGS				BENZYL-BENZOATE ADMINISTERED BY MOUTH	DATE 1923	BLOOD PRESSURE IN MM. MERCURY WITH AND WITHOUT REST					DIAGNOSIS AND REMARKS
	BLOOD N.P.N.	URINE P.S.P. %	URINE ALBUMIN	URINE CASTS			0 REST	10 MIN. REST	20 MIN. REST	30 MIN. REST	40 MIN. REST	
VII Cont'd		42	2 plus	(1.015)		6-22	192-116					
						6-23	186-112	160-108	156-106	156-106		
						7-	142-90	136-88	150-106	152-104		
						8-16	160-108	156-106				
						2-23	226-126	200-114	188-110	188-110		Arteriosclerosis
VIII				0	25 m. q.i.d.	2-24	212-116	188-108	188-108	188-110		
Female	31.2				30 m. q.i.d.	2-26	218-122	204-116	192-114	190-114		Angina pectoris
white		48	0	(1.015)	"	2-27	222-122	190-114	188-112	188-110		
age 48					Discontinued	3-6	220-130		188-108	188-108		
						4-12	222-114		180-106	186-112		
IX	35.8	46				3-7	258-140	230-130	230-132	228-130		Arteriosclerosis
Female					25 m. q.i.d.	3-8	254-136	226-128	224-128	226-130		Chronic interstitial nephritis
white					30 m. q.i.d.	3-10	248-138	236-136	226-130	226-128		
age 55			FT	0	"	3-12	244-124	222-128	224-126	226-124		
				(1.012)	"	3-14	246-126	220-124	222-124	222-126		3-17 Acute bronchitis and pharyngitis
	30.3				"	3-17	236-120	202-114	200-114	200-114		
		46			"	3-19	232-120	204-114	204-114	202-114		
					"	3-21	240-126	238-120	228-124	212-124		
					"	3-23	228-116	208-110	210-114	212-114		
					Discontinued	3-26	232-120	202-118	220-122	212-120		
						4-2	238-130	202-118	208-118	212-120		
						4-10	230-126	214-116	208-116	208-116		

TABLE I—CONT'D

CASE NO.	LABORATORY FINDINGS				BENZYL-BENZOATE ADMINISTERED BY MOUTH	DATE 1923	BLOOD PRESSURE IN MM. MERCURY WITH AND WITHOUT REST					DIAGNOSIS AND REMARKS
	BLOOD N.P.N.	URINE P.S.P. %	URINE ALBUMIN	URINE CASTS			0 REST	10 MIN. REST	20 MIN. REST	30 MIN. REST	40 MIN. REST	
X	47.6	27	4 plus	Many		3-8	210-140	200-136	190-128	190-128		Chronic interstitial nephritis
Male		42	"	H.; SG;		3-9	204-136	198-136	194-134	198-136		Arteriosclerosis
white			"	& LG		3-12	212-136	198-130	198-128	196-126		3-26 Large numbers of red and
age 46		25	"	"		3-13	198-130	192-128	192-130			white blood cells in urine.
	52.8		"	"		3-14	186-124	186-124	190-126	192-126		Sent to Genito-Urinary Clin-
	U. 4.5		"	"	25 m. q.i.d.	3-15	190-126	186-124	188-124	188-124		ic. During cystoscopy de-
	48.7		"	"	30 m. q.i.d.	3-17	188-124	184-120	186-122	188-124		veloped acute edema of lungs.
	U. 5.5		"	"	"	3-19	180-120	178-118	172-114	174-116		Later discharged from hos-
	C. 1.48		"	"	"	3-21	180-122	180-122	178-120	176-12(		pital. Death notice in news-
	50		"	"	"	3-23	182-12(	174-117	187-120	180-120		paper 3 months later. Cause
	U. 5.7				"	3-26	190-124	182-120	174-116	178-118		of death unknown. Eight
	C. 2.3											examinations of urine showed
												the presence of albumin vary-
												ing in amounts between 1
												gram and 1.8 gram per liter.
												Sp. gr. varied between 1.013
												and 1.040
XI	35.8		0	H.								Chronic interstitial nephritis
Female				(1.018)		3-9	244-136	238-128	242-130			3-15 Extra systoles
colored		41				3-10	236-126	230-124	222-118	222-124		E.K.G. Right Ventricle. Ex-
age 48						3-12	232-130	202-124	202-120	206-118		tra systoles P.R. interval 0.2
					25 m. q.i.d.	3-13	236-124	218-120	228-124	226-124		seconds
					30 m. q.i.d.	3-15	240-130	238-126	228-124	230-126		
					"	3-16	240-130	238-130	230-124	234-126		
					"	3-19	256-128	236-124	234-124	230-120		
					Discontinued	3-21	260-136	234-130	230-122	230-122		
					"	3-23	250-126	238-120	228-116	230-116		
						3-28	240-118	228-112	222-110	222-110		

TABLE I—CONT'D

CASE NO.	LABORATORY FINDINGS				BENZYL-BENZOATE ADMINISTERED BY MOUTH	DATE 1923	BLOOD PRESSURE IN MM. MERCURY WITH AND WITHOUT REST					DIAGNOSIS AND REMARKS
	BLOOD N.P.N.	URINE P.S.P. %	URINE ALBUMIN	URINE CASTS			0 REST	10 MIN. REST	20 MIN. REST	30 MIN. REST	40 MIN. REST	
XII			plus	0		6-26	210-112	190-108	190-106			Arteriosclerosis
Male						6-27	224-106	190-104	180-100	180-100		7-13 Has been hiccoughing
white					25 m. q.i.d.	6-28	230-112	190-106	186-104	186-104		every 5 to 10 seconds for
age 61					30 m. q.i.d.	6-30	200-106	186-102	182-100	180-100		past two days
					"	7-2	230-110	196-104	190-102	192-100		7-17 Severe bronchitis and
	40		VFT	0	"	7-5	206-106	190-102	184-102	184-102		slightly jaundiced Temp. 99.5
		37	(1.015)		"	7-7	218-108	200-104	188-100	184-102		F. Pulse 72
					"	7-11	208-106	186-102	188-104	188-104		
					Discontinued	7-13	204-104	177-100	176-98	194-104		
						7-17	202-100	168-98	156-86	160-88		
						7-18	202-100	168-98	156-86	160-88		
						7-23	192-98	156-88	163-94	162-94		
XIII			0			6-29	200-110			170-102		
Female	43.5	66		0(1.025)D		7-2	200-110	186-106	180-102	180-102		
white			0	0(1.010)N	25 m. q.i.d.	7-3	200-106	184-106	176-104	170-104		
age 41			0	0(1.019)	30 m. q.i.d.	7-5	198-110	178-108	176-104	174-104		
	40.8		0	0(1.019)	"	7-9	204-108	194-108	196-108	194-104		
	U. 3.7				Discontinued	7-11	214-114		180-104	188-112		
	C. 1.7					7-17	232-112	202-108		220-116		
XIV	36		plus	0		7-3	190-130	170-120	168-118	168-118		Hemiplegia, old, right
Male			T	0(1.016)D		7-5	170-126	156-116	158-112	156-116		Chronic prostatitis
colored		65	plus	0(1.012)N		7-6	192-140	170-132	170-130			
age 47			T	H.								
				(1.017)D	25 m. q.i.d.	7-7	170-126	154-116	158-11	166-122	164-120	Chronic interstitial nephritis
			VFT	(1.015)N	"	7-9	168-124	174-120	168-118	164-118		7-9 Headache
					"	7-12	192-132	174-122		172-120		
					"	7-14	192-122	168-114		162-116		7-14 Vertigo and headache
	41.7	61	plus	(1.014)D	"	7-18	180-124	174-122	172-122	172-120		
	U. 4.2		T	(1.021)N	Discontinued	7-24	178-120		170-120	172-120		

TABLE I—CONT'D

CASE NO.	LABORATORY FINDINGS				BENZYL-BENZOATE ADMINISTERED BY MOUTH	DATE 1923	BLOOD PRESSURE IN MM. MERCURY WITH AND WITHOUT REST					DIAGNOSIS AND REMARKS
	BLOOD N.P.N.	P.S.P. %	ALBUMIN	CASTS			0 REST	10 MIN. REST	20 MIN. REST	30 MIN. REST	40 MIN. REST	
XV			plus	0		7.9	266-160	252-154		232-150		Chronic interstitial nephritis
Female	32.5				25 m. q.i.d.	7-10	256-156	232-148	226-150	226-146		
colored	U. 3.2	59			30 m. q.i.d.	7-13	260-156	236-142	246-146	240-142		
age 27					Discontinued	7-19	256-160	252-154	248-152	242-140		
	34.4	36	plus			7-21	266-160	246-156	246-158	248-160		
	U. 4.7		(1.017)D	0		7-24	260-166	250-160	246-160	240-158		
			(1.004)$^{T}_{N}$									
XVI	35	46	plus	(1.007)		7-7	236-12(	220-118	210-114	212-114		Arteriosclerosis
Female					25 m. q.i.d.	7-10	234-120	216-114	204-114	206-116		
colored			VFT	0(1.006)	"	7-13	236-118	202-110	208-112			
age 63	40	50	plus	0(1.008)N		7-18	234-120	204-108	204-110	204-108		
	U. 3.6		T	0(1.019)D	Discontinued	7-21	212-120	180-108	188-106	188-106		
						7-24	230-112	210-98	210-104	210-98		

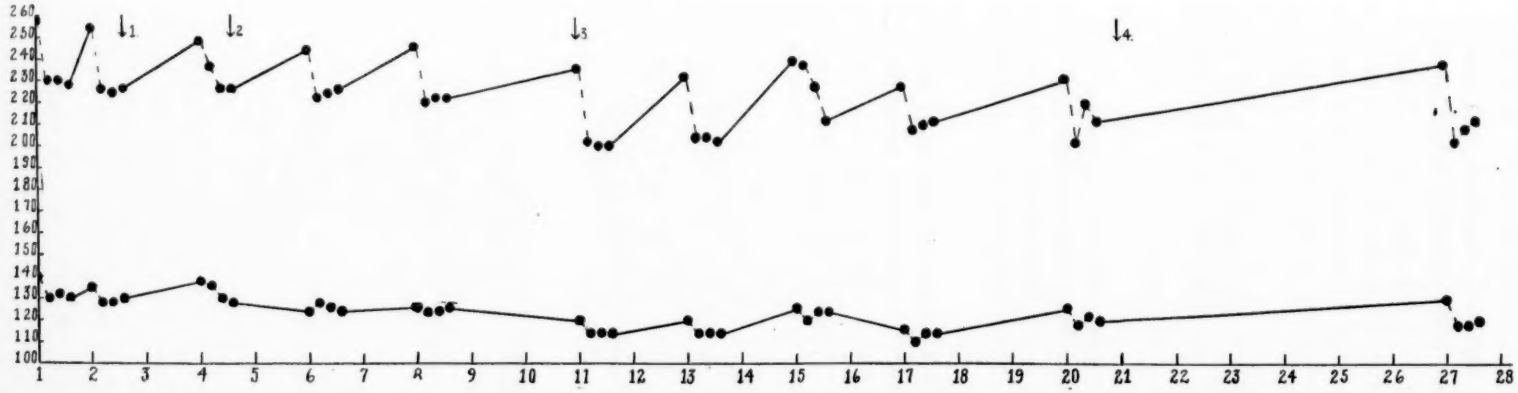

Fig. 3.—Patient IX. Same as Fig. 2. (*1*) Twenty-five drops benzyl-benzoate four times a day. (*2*) Dose increased to 30 drops four times a day. (*3*) Patient developed acute bronchial trouble. (*4*) Benzyl-benzoate discontinued.

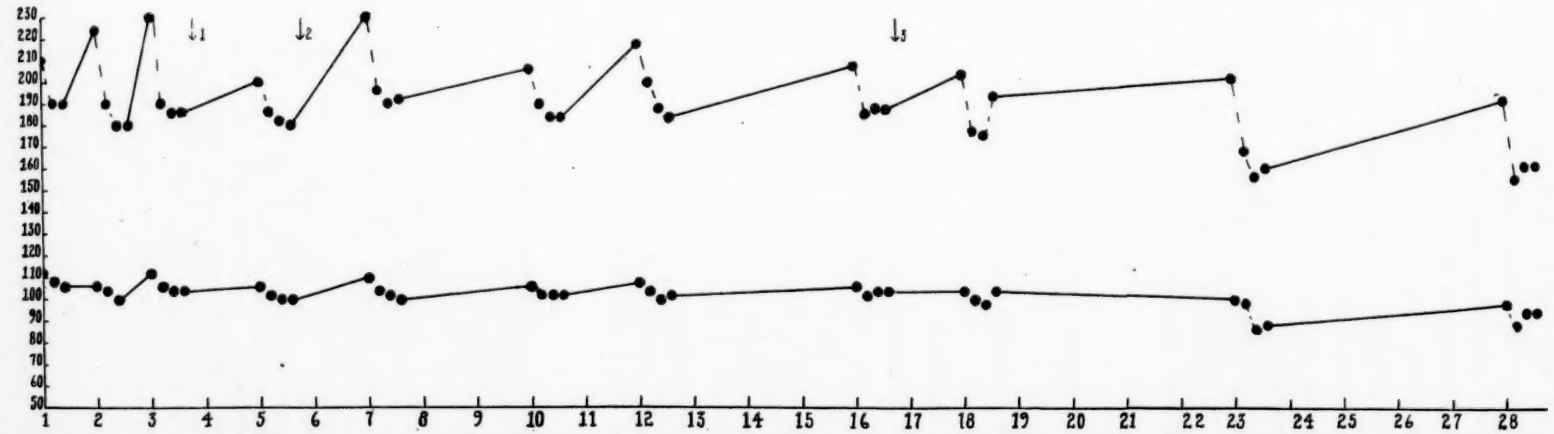

Fig. 4.—Patient XII. Same as Fig. 2. (*1*) Benzyl-benzoate, 20 per cent alcohol solution, 25 drops four times a day. (*2*) Dose increased to 30 drops four times a day. (*3*) Patient had been hiccoughing for 48 hours and benzyl-benzoate discontinued.

As said before, a fall of blood pressure was noted in one case (II). In this case the blood pressure was falling before benzyl-benzoate was given. During administration the patient became nauseated, vomited and was required to go to bed. She developed acute bronchial trouble terminating in pneumonia. Such acute infections in themselves were observed to lower the blood pressure in our hypertension cases. (See Cases I, IV, IX, XII.) In this instance as in the other cases we are inclined to attribute the reduction in blood pressure to the acute infection rather than to the effect of benzyl-benzoate.

From these results we are forced to conclude that benzyl-benzoate given in "therapeutic" doses over a long period of time in arterial hypertension cases has no effect upon blood pressure.

SUMMARY

1. Blood pressure readings, to be consistent from day to day and to be of value in such study as this, must be taken after the patient has rested at least 20 minutes. Rest markedly reduces the blood pressure in hypertension cases.

2. Benzyl-benzoate taken in 25- to 30-drop doses (20 per cent in alcoholic solution) has no immediate depressant effect upon blood pressure.

3. Benzyl-benzoate taken in 30-drop doses four times a day for four to eighteen days has no effect upon the blood pressure in patients suffering with hypertension.

4. We believe that benzyl-benzoate has no action upon the blood vessels when taken by mouth and that it should have no place in the physician's armamentarium in the treatment of the above condition.

We wish to acknowledge our indebtedness to Dr. A. M. Ecklund and Miss Charlotte Ratherd for the laboratory findings shown in Table I.

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A BACTERIOLOGIC STUDY OF EXTIRPATED TONSILS*

By Louis A. Julianelle, Ph.D., Philadelphia, Pa.

BECAUSE of the important rôle played by the tonsils in focal infections, it is of interest to place on record the bacteriologic findings in cultures made from the crypts of extirpated tonsils. Similar studies have recently been made by other investigators and have furnished valuable data for correlation with the findings in cultures of material from the surface of tonsils *in situ*. The work of Pilot and Davis[1] has shown that cultures from the crypts of excised tonsils yields a higher incidence of certain types of bacteria than was obtained by cultures of material from the surface of the same tonsils immediately before tonsillectomy.

In this study no attempt has been made to gather data for the comparison of findings in throat cultures before or after operation with the findings in cultures made from the interior of extirpated tonsils. It was our object merely to record the different types of organisms cultivable from tonsils which had been removed because of hypertrophied or septic conditions. The survey includes 147 patients. Material for culture was obtained from the depths of the crypts or from apparent purulent foci, after bisecting the tonsil with a sterile knife. This was streaked on the surface of beef-infusion blood agar (PH 7.8) and incubated at 37° C. for 24 hours, when the different types of colonies developing were picked and studied for identification.

By crediting the different types of bacteria found in a pair of tonsils to the particular patient, the incidence of organisms found may be summarized as shown in Table I.

Table I

Organisms Found in Culture of 147 Pairs of Extirpated Tonsils

ORGANISM	NUMERICAL INCIDENCE	PERCENTAGE INCIDENCE
Streptococcus Hemolyticus	133	90.4
Staphylococci	92	62.5
Streptococcus Viridans	46	31.2
M. Catarrhalis	29	19.7
B. Influenzae	25	17.0
Pneumococcus	13	8.8
B. Mucosus	8	5.4
B. Diphtheriae	6	4.0
Nonhemolytic Streptococci	2	1.3

In eight instances pure cultures were obtained; seven were hemolytic streptococci and one was streptococcus viridans. Five of the thirteen strains of pneumococcus were typed and these were found to be type II (a), two strains, and type IV, three strains.

*From the Bacteriological Laboratory of the Philadelphia General Hospital.
Received for publication Aug. 30, 1923.

The percentage incidence of the various bacteria reported here is somewhat lower than that of Pilot[2] and his co-workers, but is in fair agreement with that of Caylor and Dick.[3]

DISCUSSION

The most noteworthy feature of this study is the evidence it offers in favor of the carrier possibility of the tonsils. Nichols and Bryan,[4] Tongs,[5] Pilot and Davis,[1] Simmons and Taylor,[6] and Van Dyke[7] are in accord that in the case of streptococci the incidence is considerably less in tonsillectomized than in tonsil-bearing throats. Tongs states that "Complete tonsillectomy appears to be followed in most cases by the absence of hemolytic streptococci from the throat." Here we have tangible evidence that the tonsil is an actual focus of streptococcus infection, hemolytic, viridans, and nonhemolytic, with the hemolytic occurring in predominating numbers. These organisms were isolated from within the tissue of diseased tonsils, so that it is reasonable to assume that they were concerned in part in the production of the abnormal condition. The carrier condition is perhaps best witnessed in those instances where pure cultures were obtained.

The presence of diphtheria bacilli has been reported by Dwyer and Gignoux,[8] Ruh, Miller, and Perkins,[9] Brown,[10] Ballantyne and Cornell,[11] and Pilot.[12] It has been the experience of Pegler,[13] Friedberg,[14] Ruh, Miller and Perkins,[9] and others that the removal of tonsils in such instances usually eliminates the carrier condition. The table presented shows that B. diphtheriae was present in 4 per cent of the tonsils studied. No tests for virulence were made so that it can be stated that their presence was only suspiciously abnormal. Of the diphtheria bacilli isolated from normal throats Pennington[15] found 14 per cent virulent, Van Sholly[16] 1.8 per cent and Pilot[12] 16.66 per cent.

The staphylococci and M. catarrhalis are usually regarded as normal inhabitants of the mouth and throat and except in rare instances are of no significance. The influenza bacilli were found present in a percentage which is normal during an interepidemic period. The frequency of pneumococci was also within normal limits and the strains that were typed point to no abnormal condition. None of these four organisms were found in pure culture or in predominating numbers, so that their presence is explainable on a lowered resistance of the tonsil, rather than a true invasiveness on their part.

It is difficult to state the position of B. mucosus in this study. The organism was found in a small percentage of cases and except once with a diphtheria bacillus and once with a streptococcus viridans, in conjunction with hemolytic streptococci. It can cause both acute and chronic conditions, particularly of the lung, but we are inclined to believe that its status here was more of the nature of a temporary inhabitant as a saprophytic organism.

SUMMARY

A bacteriologic study has been made of tonsils removed because of hypertrophy or septic conditions. The findings have been presented and discussed in brief.

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THE EXCRETION OF CREATININE IN URANIUM NEPHRITIS*

By Ralph H. Major, M.D., Kansas City, Kansas

In a recent study of the excretion of creatinine following intravenous injection of this substance,† certain differences were pointed out between the results obtained in normal individuals and in patients suffering from renal disease. In normal controls and in patients with no evidence of kidney disease, the intravenous injection of creatinine is followed by a marked increase in the urinary excretion of creatinine, while in patients suffering from chronic nephritis, this increase was either slight or entirely absent and at times an actual decrease in excretion was noted.

These observations suggested very strongly that while the kidneys in health respond promptly to an increase of blood creatinine by excreting this excess, in nephritis such an increase in blood creatinine is not taken care of, and produces at times a partial paralysis in the creatinine excreting ability of the diseased kidneys. This observation is of especial interest because of the unusual ability of the normal kidneys to concentrate and to excrete creatinine and because of the generally accepted view that this ability is one of the last functions of the kidneys to fail in renal disease. It seemed therefore desirable to study this subject further by experimental work under carefully controlled conditions.

Rabbits were used in these experiments and each animal received 1 gm. of creatinine dissolved in 3 c.c. of distilled water. The blood creatinine was estimated just before injection, and one-half hour, one hour and two hours after injection. The urine was collected for one hour before injection and for one hour and two hours following injection, and creatinine determinations made on each specimen. The animal was catheterized just before the experi-

*From the Department of Internal Medicine, University of Kansas School of Medicine. Received for publication, October 27, 1923.

†The Use of Creatinine as a Test of Renal Function, Jour. Am. Med. Assn., 1923, lxxx, 384.

ment and each specimen was obtained by catheter. The method of Folin was used for the creatinine determinations.

The effect of the intravenous injection of creatinine upon each animal was first studied and then the animal received an intravenous injection of uranium nitrate .005 gm. per kilogram of body weight. Twenty-four hours later the animal was again given an intravenous injection of creatinine and its excretion studied.

An examination of Table I shows higher values for the blood creatinine after the uranium injections as would be expected. The percentage increase in the blood creatinine following the injection of creatinine, is however, about the same for the animals injected with uranium as for the normal ones.

The study of the urinary excretion in Table II, however, shows a marked difference between the normal animals and those injected with uranium. The normal animals respond to an intravenous injection of creatinine with a greatly increased output of creatinine, the average excretion during the first and second hour after injection being respectively six times and five times that of the hour preceding injection. Twenty-four hours after the animal had received uranium nitrate, the average total excretion of creatinine was only slightly increased one hour after injection and two hours after was decreased. Five of the seven animals showed less excretion of creatinine during

TABLE I

THE EFFECTS OF INTRAVENOUS INJECTION OF CREATININE UPON THE BLOOD CREATININE IN NORMAL AND IN NEPHROPATHIC ANIMALS

	BEFORE INJECTION WITH URANIUM				24 HOURS AFTER INJECTION WITH URANIUM			
	BLOOD CREATININE MG. PER 100 C.C.				BLOOD CREATININE MG. PER 100 C.C.			
	BEFORE INJECTION	½ HOUR AFTER INJECTION	1 HOUR AFTER INJECTION	2 HOURS AFTER INJECTION	BEFORE INJECTION	½ HOUR AFTER INJECTION	1 HOUR AFTER INJECTION	2 HOURS AFTER INJECTION
1.	1.3	2.6	2.3	1.6	2.2	8.4	6.9	6.6
2.	1.4	6.0	4	3.4	5.4	8.1	7.8	7.5
3.	1.5	7.0	4.9	4.4	6	9.6	12	9.8
4.	1.6	5.9	3.9	2.9	4.3	8.0	7.2	8.0
5.	1.6	3.8	2.7	2.3	2	6.8	4.5	3.2
6.	1.6	2.6	2.1	1.7	5.3	8.0	8.0	9.6
7.	1.4	4.9	3.9	3.2	3.8	6.0	5.0	5.0

TABLE II

THE EFFECTS OF INTRAVENOUS INJECTION OF CREATININE UPON THE CREATININE EXCRETION IN NORMAL AND IN NEPHROPATHIC ANIMALS

	BEFORE INJECTION WITH URANIUM						24 HOURS AFTER INJECTION WITH URANIUM					
	CREATININE IN URINE						CREATININE IN URINE					
	BEFORE INJECTION		1 HR. AFTER		2 HOURS AFTER		BEFORE INJECTION		1 HR. AFTER		2 HOURS AFTER	
	MG. PER 100 C.C	TOTAL	MG. PER 100 C.C	TOTAL	MG. PER 100 C.C	TOTAL	MG. PER 100 C.C	TOTAL	MG. PER 100 C.C	TOTAL	MG. PER 100 C.C.	TOTAL
1.	125	5	...	..	992	50	200	8	100	3	104	3
2.	160	8	...	..	1000	60	32	0.3	13	.07	11	.06
3.	189	8	665	33	976	29	17	3	16	.08	8	.06
4.	147	4	992	59	620	12	38	8	51	10	46	2
5.	204	6	780	19	534	16	71	2	280	15	209	3
6.	43	4	801	24	320	13	59	.6	5	.02	5	.02
7.	124	6	1165	58	741	30	54	2	12	.06	...	...

the first and second hour after injection than during the hour before injection. A chart drawn from the average creatinine excretion is shown in Fig. 1.

The depression of creatinine excretion following the intravenous injection of creatinine is apparently closely related to the severity of the renal lesion. This is indicated by the fact that the animals showing the most marked de-

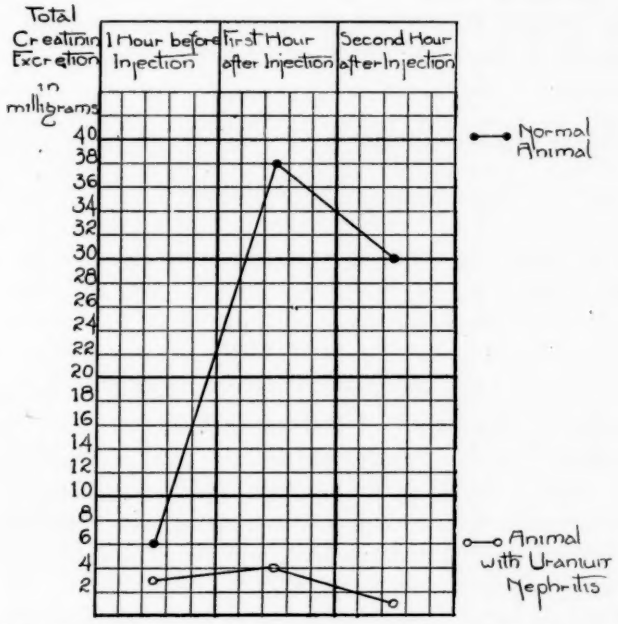

Fig. 1.—Curve showing creatinine excretion in normal and nephropathic animals following intravenous injection of 0.1 gm. creatinine.

pression were those having the highest values for blood creatinine and is also shown by the results obtained in three animals detailed in Table III.

These three animals after a study of their creatinine excretion were given .005 gm. of uranium nitrate per kilo body weight intravenously and

TABLE III

THE EFFECTS OF INTRAVENOUS INJECTION OF CREATININE UPON THE CREATININE EXCRETION IN ANIMALS SHOWING INCREASING DEGREE OF RENAL INSUFFICIENCY

		RABBIT NO. 8			RABBIT NO. 9			RABBIT NO. 10		
		AMT. URINE IN C.C.	CREAT. MG. PER 100 C.C.	TOTAL CREAT.	AMT. URINE IN C.C.	CREAT. MG. PER 100 C.C.	TOTAL CREAT.	AMT. URINE IN C.C.	CREAT. MG. PER 100 C.C.	TOTAL CREAT.
Normal	1	11	58	6	10	80	8	12	42	5
	2	10	309	31	13	190	25	12	266	31
	3	15	69	10	11	133	14	14	106	15
Sixteen hours	1	15	80	12	13	14	2	15	47	7
after Uranium	2	13	210	27	13	50	7	20	43	9
Inject.	3	13	13	13	13	87	11	13	61	8
Forty hours	1	5	15	.8	12	+	+	12	21	2
after Uranium	2	14	0	0	9	0	0	8	0	0
Inject.	3	11	0	0	9	0	0	6	0	0

1. One hour before injection of 0.1 gm. creatinine.
2. One hour after injection.
3. Two hours after injection.

then injected with creatinine sixteen hours later, and again forty hours later. Sixteen hours after injection with uranium nitrate the animals had a relatively mild kidney lesion and the excretion of creatinine shows only a moderate diminution. Forty hours after injection with uranium nitrate the animals had a very marked nephritis and the injection of creatinine intravenously produced a complete suppression of creatinine excretion.

The increase in severity of the kidney lesion in these animals is shown by the changes in the blood chemistry in Table IV.

TABLE IV

INCREASE IN BLOOD CREATININE WITH INCREASING SEVERITY OF RENAL LESION

	BLOOD CREATININE			BLOOD UREA		
Rabbit	No. 8	No. 9	No. 10	No. 8	No. 9	No. 10
Normal	2.0	1.8	2.0	18	14	17
16 hrs. after Uranium	2.7	2.5	3.8	26	25	34
40 hrs. after Uranium	9.0	9.6	10.8	106	129	144

SUMMARY

The intravenous injection of 0.1 gm. creatinine in normal rabbits is followed by a prompt and marked increase in the creatinine excretion.

In rabbits rendered nephropathic by the administration of uranium nitrate this marked increase in excretion does not appear.

As the uranium nephritis becomes marked, the intravenous injection of creatinine results first in a depression and later in a complete suppression of creatinine excretion.

COMPARISON OF WASSERMANN AND PRECIPITATION REACTIONS IN VARIOUS STAGES OF SYPHILIS*

BY M. A. WILSON AND ROSE M. NEDLEY, NEW YORK, N. Y.

IN connection with our studies on the standardization of the Wassermann test, we have reviewed the literature on the various precipitation tests for the diagnosis of syphilis. It had been thought that the numerous sources of error in the Wassermann procedure, due to the variation of complement in its relation to fixability and to hemolysis, might be eliminated by the simpler reaction of precipitation. However, our search in the literature gave us no alluring promise of a speedy release from the perplexities that accompany even the best regulated of the Wassermann technics. The publications on precipitation tests for syphilis, from the time of Fornet and Schereschewsky[1] in 1907 up to the more recent work of Meinicke,[2] Sachs and Georgi[3] and others have been so thoroughly reviewed by Olitsky and Olmstead,[4] Kilduffe,[5] Kahn[6, 7, 8] and others[10, 11] that no description of those methods is needed at this time. In regard to the value of the tests described by the above-named authors the concensus of opinion is that none of the precipitation procedures

*From the Bureau of Laboratories, Department of Health, New York City.

has proved to be more delicate or more specific than the Wassermann test. As the published laboratory studies of precipitation methods were made by workers of known ability a repetition by us of their investigations seemed to be unnecessary.

It was not until the results obtained with Kahn's modification of Meinicke's method were made known that a useful test seemed to have been devised. However, the conclusions of workers[5, 10, 11] with Kahn's method, although cordial to the idea of eventually evolving a technic that would supersede the Wassermann test, have been very conservative in suggesting that the new precipitation test be substituted for the Wassermann. It has been recommended for a check, rather than as a substitute.

In the routine work of the larger municipal laboratories, where from 350 to 500 serums must be tested daily, to adopt a new test that would be merely a corroborative check and which would require the whole time of one technician, or more, would seem to us to be a greater outlay of salary expense than the benefits of such a check would justify. It is our opinion that any of the carefully controlled Wassermann procedures will give the maximum information in regard to the reacting substance in syphilitic serums. If the precipitation test could be made to give a reaction earlier in the disease, or, if the precipitation curve of repeated bleedings would run a more even course than the Wassermann curve, such a check would be of value.

From our preliminary study* we chose, first, the question of the relation of the precipitation test to the stage of disease, and, second, the comparison of Wassermann and precipitation curves. We followed Kahn's technic in the most minute detail, as given in his publications[6, 7, 8] and in personal communications. Our results with his earlier technic showed his test to be comparable with the Wassermann test when the serums were tested within twenty-four hours after the bleeding of the patient. With older serums the precipitation test was inferior to the Wassermann. The curves in our first study were all of older serums, and therefore the precipitation curves compared unfavorably with the Wassermann curve.

In our further studies we have followed Kahn's later technic.[12]

Comparative Wassermann and Kahn tests have been made upon serums at periods of from twenty-four hours to four days after the bleeding of the patient. In this series of tests the age of the serum did not interfere with the precipitation reaction. The Wassermann tests were made by the Wassermann Laboratory of the New York City Health Department, and the Kahn tests were made by technicians from the Serologic Laboratory of the same department. The Wassermann and Kahn tests were made on the same day, all the technicians working in the same room.

The serums in the series reported in this paper were sent to the Wassermann laboratory from various sources, namely, from clinics, hospitals, orphanages and physicians. As is usual in such cases no clinical data accompanied the specimen of blood. Later, some of the clinicians sent us their clinical data, thus enabling us to make a classified table. One clinician who was reluctant to put the clinical data into our hands gave us his opinion of

*Read before the Association of Immunologists at Boston, March, 1923.

the precipitation test. A report of the Wassermann and the precipitation tests on each serum was sent to the clinicians before we asked them for clinical data.

In compiling the laboratory tests and clinical data we selected cases from the Venereal Disease Department of the Kingston Avenue Hospital, Department of Health, with a view to determining, first, the relation of the precipitation test to the stage of disease, and, second, to compare the Wassermann and precipitation curves of cases under treatment. In answer to the first question this service gave us too few cases of early syphilis to draw conclusions. Of 117 cases there were only ten of early syphilis and 80 per cent of those were positive by both tests. One case was positive by Kahn and negative by Wassermann and one case was doubtful by both tests. Of 29 cases of serologic and latent syphilis, 23 cases, or 80 per cent, were positive by both Wassermann and Kahn, 7 cases were positive by Kahn and negative by Wassermann, and one case was doubtful by both tests. The summary of those tests is given in Table I.

TABLE I

SHOWING COMPARISON OF WASSERMANN AND KAHN DIAGNOSES IN VARIOUS STAGES OF SYPHILIS

CLINICAL DIAGNOSIS	STAGE OF DISEASE	NUMBER OF CASES	DIAGNOSES BY WASSERMANN AND KAHN TESTS*			
			WASS. POSITIVE KAHN POSITIVE	WASS. POS. KAHN NEG.	KAHN POS. WASS. NEG.	BOTH TESTS NEGATIVE, OR DOUBTFUL
Syphilis	Primary	1	1			
	Active Secondary	3	2			1
	Serologic	10	8		2	
	Latent	2	2			
Syphilis: and Gonorrhea:	Latent, or Serologic Chronic, or Clinical	6	5		1	
Syphilis: and Gonorrhea:	Latent, or Serologic Chronic, or Clinical	17	13		5	1
Syphilis: and Gonorrhea:	Congenital Clinical	1	1			
Gonorrhea:	Chronic, or Clinical	77	5	3	3	63
Totals		117	37	3	12	65

*The diagnoses shown in Table I represent the height of reaction in the curve. Most of the cases were tested at weekly intervals over a period of three weeks to seven weeks.

For our second question, i.e., comparison of Wassermann and precipitation curves, we made a classified chart of the curves of the above 117 cases. For our Table II we selected eighteen of those cases that are fairly representative of the whole.

An examination of the curves shown in Table II shows that in the case of primary syphilis the Wassermann and precipitation curves ran the same course for five weeks, although the Kahn precipitation reaction was weaker than the Wassermann reaction. In two cases of active secondary syphilis

complicated by gonorrhea (Cases 9 and 10) the trend of the curves was the same, but the precipitation reaction was stronger than the Wassermann. In latent and serologic syphilis the Wassermann curve was irregular in more cases than was the Kahn precipitation curve.

From one clinic we received serums from 363 cases, in which the Wassermann and precipitation tests agreed in 77 per cent of the cases. Of the disagreements: 79 per cent were Kahn positive and Wassermann negative or doubtful, and 21 per cent were Wassermann positive and Kahn negative, or doubtful. We had hoped to be able to make a classified chart of this list of cases in order to have further evidence in regard to the relation of the

TABLE II

COMPARISON OF WASSERMANN AND PRECIPITATION CURVES

CLINICAL DIAGNOSIS	STAGE OF DISEASE	CASE NUMBER	WASSERMANN AND PRECIPITATION CURVES								
			TEST	1ST WEEK	2ND WEEK	3RD WEEK	4TH WEEK	5TH WEEK	6TH WEEK	7TH WEEK	8TH WEEK
Syphilis	Primary	1	Wass.	4	4	4	2	±			
			Kahn	2	2	2	±	±			
	Active Secondary	2	Wass.	4	4	4					
			Kahn	4	4	4					
		3	Wass.	2	4			4	+	±	
			Kahn	4	4			4	4	3	
		4	Wass.	−	±						
			Kahn	−	−						
	Latent	5	Wass.	+	−	2					
			Kahn	3	4	4					
		6	Wass.	+		±	±	−	2	−	
			Kahn	3		4	4	4	4	2	
	Serologic	7	Wass.	4	3	−	−	−	−	−	
			Kahn	4	4	4	3	3	3	4	
Syphilis and Gonorrhea	Serologic	8	Wass.	2	±	4					
			Kahn	2	3	4					
	Active Secondary	9	Wass.	+	−	2					
			Kahn	3	3	4					
	Active Secondary	10	Wass.	3	−	−					
			Kahn	4	3	3					
	Serologic	11	Wass.	4	4	4			4	±	
			Kahn	4	4	4			4	+	
	Serologic	12	Wass.	4	4		4	4			4
			Kahn	4	3		3	4			2
	Serologic	13	Wass.	±	3	4					
			Kahn	3	2	2					
	Serologic	14	Wass.	±	2	4		±	2		
			Kahn	2	3	3		3	2		
	Serologic	15	Wass.	4	4	4	4	2	−		
			Kahn	4	3	4	4	4	4		
	Serologic	16	Wass.	4	4	4	±		3	+	
			Kahn	2	4	4	4		3	4	
	Serologic	17	Wass.	3	2	±	2	−	−		
			Kahn	4	2	2	+	+	+		
	Serologic	18	Wass.	4	4	+	−	2	4		2
			Kahn	4	4	4	4	2	3		2

precipitation test to the stage of disease. However, the clinician in charge of this service has withheld the clinical data, so that we were unable to present a chart in this paper. The clinician has given us his opinion of the Kahn precipitation test, as follows: First, that the Kahn precipitation reaction appears to be more sensitive than the Wassermann in cases of active syphilis; second, if the Kahn precipitation reaction is negative, the case is probably nonsyphilitic; third, that he thinks a great deal more study of the precipitation reaction must be made in relation to the condition of the patient, before we are justified in accepting the test as a routine diagnostic measure. This clinician is at present selecting cases for our study and will give us his hearty cooperation in the future.

For controls of the specificity of the Wassermann and Kahn precipitation tests, we selected serums from cases that might be expected to be nonsyphilitic, such as children in orphanages, children's clinics, etc. The results of those tests are shown in Table III.

TABLE III

WASSERMANN AND PRECIPITATION TESTS ON CONTROL CASES

INSTITUTION	NUMBER OF CASES	WASSERMANN AND PRECIPITATION TESTS			
		WASSERMANN NEGATIVE KAHN NEGATIVE	WASS. POS. KAHN POS.	KAHN POS. WASS. NEG.	WASS. POS. KAHN NEG.
A	351	334	12	4	1
B	20	18	1	1	
C	10	10			
D	9	9			
Totals	390	371	13	5	1

Of 390 cases from orphanages, nurseries, children's hospitals, etc., 371, or 95 per cent, were negative by both Wassermann and Kahn; 13 cases, or 0.3 per cent, were positive by both tests. Of these latter six were clinically diagnosed as congenital syphilis; seven were normal maternity cases, or had no history. Of the five cases positive by Kahn and negative by Wassermann all were maternity cases of normal deliveries—mothers having no history of syphilis. The one case positive by Wassermann and negative by Kahn was clinically diagnosed as congenital syphilis.

It is claimed by some clinicians that serums from tubercular patients will give a nonspecific Wassermann reaction. With that question in mind we made comparative Wassermann and Kahn tests of 153 cases of pulmonary tuberculosis. Of these: 137, or 89 per cent, were negative by both tests; seven cases were positive by both tests; five were positive by Kahn and negative by Wassermann and four were positive by Wassermann and negative by Kahn's method. Of the twelve cases that gave positive precipitation reactions, seven had history of chancre; the others denied syphilitic history.

A study of the Kahn precipitation test in relation to the clinical data, as shown in our tables, leads us to believe that our results are corroborative evidence of the claims made by Kahn and others for that test.

We wish to express our appreciation of the cooperation of Mr. Koopman of the Wassermann Laboratory, Dr. Chargin, of Manhattan Clinic, Drs. Campbell and von Bose, of Kingston Avenue Hospital.

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CLINICAL SYPHILIS AND THE "STANDARD WASSERMANN (KOLMER'S)"*

By W. F. Lorenz, M.D., and W. J. Bleckwenn, M.D., Madison, Wis.

IN November, 1921, Dr. J. A. Kolmer requested us to try out the standard Wassermann that he developed and has since reported.†

It was our aim to employ Kolmer's method in a large series of cases; to parallel the examinations with a method that had been used by us for several years and especially to judge the merits of the standard proposed by a careful clinical examination wherever possible.

In our laboratory, as in practically every large laboratory, a Wassermann technic was developed during the past seven years which, in our hands, proved very satisfactory. We, therefore, decided to parallel the Kolmer method with ours.

We approached this problem as clinicians. Every case, excepting some that will be noted in the report, was seen by us personally and diagnosed from a clinical standpoint. We decided to place ourselves in the position of the clinician who might receive valuable information from serologic examinations, but the diagnosis of our cases was from a clinical, not serologic standpoint.

In passing upon Kolmer's new method one must obviously follow the technic as laid down by him and not depart in the slightest degree from the method he detailed. When this is done undoubtedly all will find that Kolmer's claims for the new method are absolutely justified. It must be recalled that he and his associates have labored with this problem for over six years, during which time they had investigated every phase of the Wassermann test and that this proposed "standard test" came only after this very painstaking effort.

CLINICAL MATERIAL

Of the 997 cases from which our conclusions are drawn, we personally examined 730. The remaining 267 cases were not seen by us but we did

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receive sufficient clinical information from reliable sources to permit their being included in our classification. The cases that we personally saw were grouped by us from a clinical standpoint and the blood for the Wassermann test was taken at the time of our examination. We had prepared beforehand a clinical sheet covering all the outstanding manifestations of syphilis. Every case examined was subjected to this routine examination which sought to bring out all the usual manifestations of syphilis from the earliest to the latest stages.

In our series we had 53 patients with chancres. Of these, one was located in the left axilla, one of the upper lip, five of the lower lip, one of the tongue, two of the scrotum and two just inside of the meatus. The remainder were of the glans, shaft, sulcus, etc. We had 31 cases that we classified as belonging to the secondary stage. In this group the most prominent manifestations were roseola, generalized adenopathy, mucous membrane lesions, headaches, alopecia, etc. Of the tertiary cases we had 279. This included 17 cases of gummata in various locations, 11 cases with aneurysm and marked aortitis, 7 cases with tertiary skin lesions and 244 that were grouped as latent syphilis, among which some had been previously treated and others had received little or no treatment but without clinical evidence of activity. Finally, we had 102 cases of syphilis of the central nervous system, of which 42 were regarded as meningovascular syphilis, 6 as clinical paresis, 32 as asymptomatic paresis, 9 as taboparesis and 13 as tabes.

COLLECTION OF MATERIAL

The blood specimen was drawn by means of a 5 c.c. Luer syringe. Before drawing blood the syringe was washed in a sterile normal saline solution. Immediately after withdrawal the specimen was placed in a small vial, sealed with a rubber stopper and then shipped by mail in a wooden mailing tube. When received at the laboratory it was centrifuged and kept in the ice box until tested which was, on the average, 24 to 36 hours after the time of taking. By following this method of shipping the blood specimens through the mails we conformed to a rather common practice and incidentally paralleled the Wassermann service which our Institute renders to physicians throughout the State of Wisconsin. The point emphasized is that all specimens were treated alike, subject to the possible changes that might result from shipment through the mails.

KOLMER METHOD

We carefully followed Kolmer's technic in every detail. During the early phase of our investigation we followed the system of dilutions that he first advocated; later, we conformed to the change which he reported as preferable. In no instance did we depart from the method outlined by Kolmer and throughout this investigation the antigen used in his method was supplied by him.

OUR TECHNIC

The Wassermann system employed by us in this comparative study has been our routine technic since 1919. We have examined over 80,000 specimens by this method. The principles underlying our technic are: the use

of varying amounts of complement so adjusted as to permit an estimate of the amount of complement fixed by a positive serum. We use a fixed amount of serum; an acetone insoluble antigen (Noguchi); the sheep hemolytic system and incubation at 37.5° C. Daily titrations are made of all elements that go into the test. We use unheated serum in which the degree of native activity is determined at the time the test is performed. Eight tubes are required for a single specimen.

As a routine, therefore, we use more tubes and material than Kolmer. Space does not permit of a detailed description of our technic. Furthermore, this seems unnecessary because we will not advocate our technic as a substitute for Kolmer's method. We wish, however, to stress the fact that our

TABLE I

TOTAL COMPARATIVE RESULTS WITH THE 3 SEROLOGIC METHODS. MATERIAL GROUPED FROM A CLINICAL STANDPOINT

TYPE	NUMBER OF CASES	BLOOD POSITIVE			BLOOD NEGATIVE		
		KOLMER	ROUTINE CHOLEST. ANTIGEN	ROUTINE NOGUCHI ANTIGEN	KOLMER	ROUTINE CHOLEST. ANTIGEN	ROUTINE NOGUCHI ANTIGEN
Primary	53	30 56%	31 58%	28 53%	23 44%	22 42%	25 47%
Secondary	31	30 97%	28 90%	27 88%	1 3%	3 10%	4 12%
Tertiary	279	197 70%	192 68%	186 66%	82 30%	87 32%	93 34%
Central Nervous Syphilis	102	78 76%	71 69%	70 68%	24 24%	31 31%	32 32%
Total Positive Cases	465	335 72%	322 69%	311 68%	130 28%	143 31%	154 32%
Total Negative Cases	518	*3	*5	*3	515	513	515
Anticomplimentary specimens	14	6	Anticomplementary		8	Anticomplementary	
GRAND TOTAL	997						

*False positive reactions in clinically negative cases.

routine technic requires a greater effort than is advocated by Kolmer. This will serve to indicate our stand in the efforts made by some to shorten, simplify and develop short-cut serology.

We had hoped that Kolmer would advocate a far more laborious method than the one he offers as a standard. We believe that a standard method should not regard time, labor or material but should be of such a character as to shoulder the responsibility that any serologic method carries. We feel that the efforts to simplify have brought about the disconcerting disharmony of reports and the clinicians' skepticism of the serologist.

DISCUSSION

In discussing the results of our comparative tests, we will refer to the Kolmer method as such, and to our routine test according to the antigen used; viz., "Noguchi antigen" will mean our routine test in which comple-

TABLE III

CASES UNDER TREATMENT—POSITIVE WITH KOLMER—NEGATIVE WITH ROUTINE
(29 of 997 cases)

CASE NUMBER	KOLMER	ROUTINE CHOLEST. ANTIGEN	ROUTINE NOGUCHI ANTIGEN	REMARKS
1897	440000	–	–	F. 30 yrs. Syphilis 6 yrs. ago; 5 courses Neo and Hg. Last 6 mos. 4 neg. bloods.
10699	344400	–	–	M. 32 yrs. Chancre 2 yrs. ago; 4 courses Neo; Blood 2-plus 6 mos. ago; rest 6 wks.
11340	220000	–	–	M. 34 yrs. Chancre 12 yrs. ago; 6 courses Neo and Hg. Last treatment 1 mo. ago; Clinically, Paresis.
11749	210000	–	–	M. 26 yrs. Chancre 6 yrs. ago; 16 Neos and Hg. Last treatment 1 wk. ago.
12357	443000	+	–	M. 43 yrs. Chancre 23 yrs. ago; 30 Neos and Hg. in past 2 yrs. 3 last bloods negative. Clinically, Cerebrospinal lues.
13007	211000	±	–	M. 40 yrs. Chancre 13 yrs. ago; 18 Neos; 30 Hg. Blood 4-plus 4 mos. ago; last treatment 2 wks. ago.
14061	444400	++	–	M. 25 yrs. Chancre 1 yr. ago; 16 Neos and Hg.
16329	444400	–	–	M. 52 yrs. Chancre 30 yrs. ago; 15 Neos and 30 Hg. in past 8 mos. Clinically, Paresis.
16775	441000	–	–	M. 29 yrs. Chancre 12 yrs. ago; 6 mos. ago blood 4-plus; 18 Neos and Hg. 2 neg. bloods last 2 wks. Clinically, Cerebrospinal Syphilis.
16786	443000	–	–	M. 34 yrs. Chancre 7 yrs. ago; 3 courses Neo and Hg. in past year. Blood 2-plus one yr. ago.
16853	444000	–	–	M. 41 yrs. Chancre 6 yrs. ago; blood 4-plus 4 mos. ago; 6 Neos; 12 Hg. last treatment 1 mo. ago.
16869	444400	–	–	M. 41 yrs. Chancre 20 yrs. ago; 15 Neos and 40 Hg. in past 2 yrs. also 8 Swift-Ellis treatments; spinal fluid neg.; Clinically, Case of Tabes.
16913	341000	–	–	M. 23 yrs. Chancre 3 yrs. ago; 20 Neos and 40 Hg. No treatment in past 6 mos. 3 neg. bloods past 3 mos.
16681	444000	±	–	M. 35 yrs. Chancre 4 yrs. ago; blood 4-plus 1 yr. ago; 14 Neos; 20 Hg. 2 neg. bloods past 2 mos.; provocative 6th da.
17102	320000	–	–	M. 30 yrs. Chancre 2 yrs. ago; 15 Neos 20 Hg. No treatment 6 mos. 3 neg. bloods.
17179	340000	±	–	M. 36 yrs. Chancre 4 yrs. ago; 3 courses Neo and Hg. in past year; blood 1-plus 3 mos. ago; neg. 1 mo. ago.
17184	442000	–	–	M. 27 yrs. Chancre denied; G. C. 3 times blood 4-plus 1 yr. ago; 3 courses Neo and Hg.
17189	343000	++	–	M. 26 yrs. Chancre 7 yrs. ago; Gumma of ribs; 3 courses Neo and Hg.
17250	411000	–	–	M. 28 yrs. Chancre 2 yrs. ago; Blood 4-plus 1 yr. ago; 15 Neos; 20 Hg. Blood 1 mo. ago negative.
17657	410000	–	–	M. 51 yrs. Chancre 30 years ago; Blood 4-plus 1 yr. ago; 12 Neos; 20 Hg. in past year. Clinically, C. S. Lues.
8127	300000	–	–	M. 37 yrs. Chancre 8 yrs. ago; 4 courses Neo and Hg. in past 2 yrs. Blood Negative 6 wks. ago.
A. R.	400000	–	–	M. 30 yrs. Chancre 10 yrs. ago; 3 courses Neo and Hg. in past year; last 2 bloods negative.
M. K.	441000	–	–	M. 31 yrs. Chancre 3 yrs. ago; 15 Neos and Hg. in past 8 mos. Last blood negative.

TABLE III—CONT'D

CASES UNDER TREATMENT—POSITIVE WITH KOLMER—NEGATIVE WITH ROUTINE
(29 of 997 cases)

CASE NUMBER	KOLMER	ROUTINE CHOLEST. ANTIGEN	ROUTINE NOGUCHI ANTIGEN	REMARKS
M. H.	341000	–	–	M. 30 yrs. Chancre 6 yrs. ago; 5 courses Neo and Hg. in past 3 years. 3 negative bloods in past 3 mos.
A. P.	440000	–	–	M. 40 yrs. Chancre 18 yrs. ago; blood 4-plus 1 yr. ago; 3 courses Neo and Hg. in past year. Clinically, Paresis
B. C.	440000	–	–	M. 32 yrs. Chancre 8 yrs. ago; blood 4-plus 2 yrs. ago. 4 courses Neo and Hg. in past 2 yrs. Last 2 bloods negative.
M. R.	410000	–	–	M. 39 yrs. Denies chancre; blood 4-plus 1 year. ago; 3 courses Neo and Hg. last blood negative.
B. D.	400000	–	–	M. 26 yrs. Chancre 4 yrs. ago; blood 4-plus 2 yrs. ago; 3 courses Neo and Hg. in past yr. Last 2 bloods negative.
20769	310000	±	–	M. 42 yrs. Chancre 12 yrs. ago; b'ood 4-plus 2 yrs. ago; 30 Neos; 40 Hg. Last 4 bloods negative.

ment titration is used, and the acetone insoluble fraction of the alcoholic ethereal extract of heart muscle as antigen. "Cholesterinized antigen" will mean the same procedure with an alcoholic extract of heart muscle to which .2 per cent of cholesterol has been added, this being half saturation.

Of the 997 cases included in this report 465 were diagnosed by us as "clinical syphilis." By "clinical syphilis" we mean cases in which the history of chancre, secondaries, and other manifestations, either present or past, are so definite as to leave no doubt as to the syphilitic infection. All of these cases, however, were not active. Two hundred forty-four of the 279 cases classified as belonging to tertiary syphilis showed no clinical evidence of activity. Many of these cases had been treated and the negative serologic findings in Table I were largely in this group.

The three serologic methods employed gave the following results in the total number of 465 cases of clinical syphilis. The Kolmer method was positive in 335 or 72 per cent; the cholesterinized antigen was positive in 322 or 69 per cent; and the Noguchi antigen positive in 311 or 68 per cent. The difference in reports would seem to be very small, i.e., 22 cases (2 per cent), if judged by these totals. This, however, is not correct because a number of cases were positive with one or two antigens and negative with the third, or *vice versa*. Such disagreements occurred in 72 cases or 7 per cent of the total number examined. These disagreements we have analyzed and shown in Table No. II, in each case giving the serologic result and the significant clinical data. Nevertheless, taking into account these differences, one is still impressed with the uniformity of results in this large number of cases. As a whole, the Kolmer method brings out more positive reactions in clinically syphilitic cases than either the cholesterinized or Noguchi antigens as used in our method.

We have compared the three methods in each of the clinical groups and

find that in the chancre stage the cholesterinized antigen tends to become positive sooner in the development of the chancre than either the Kolmer method or our method with Noguchi antigen. Of the 53 cases with initial lesions the Kolmer method was positive in 28; the cholesterinized antigen in 30 and the Noguchi antigen in 26. It is striking that all methods agreed in 20 cases in which the Wassermann reaction was negative during the chancre period and likewise all methods agreed in 25 cases that were positive; in other words, there was complete agreement in approximately 87 per cent of the cases during the chancre stage. It will also be noted that the initial lesions were positive for the dark-field in 36 or 68 per cent of the cases.

With secondary syphilis the Kolmer method was most sensitive and our method with cholesterinized antigen ranged next in order, with the Noguchi antigen least sensitive of the three. The three methods were positive in the secondary stage in the following percentages:

Kolmer	97%
Cholesterinized Antigen	90%
Noguchi Antigen	88%

It should be noted, as shown in Table I, that these differences represent only 3 cases.

In tertiary syphilis (279 cases) the Kolmer method was positive in 70 per cent; the cholesterinized antigen in 68 per cent and the Noguchi antigen in 66 per cent. This represents a difference in 11 cases of the 279 examined and shows a remarkable uniformity.

In syphilis of the central nervous system the Kolmer method was positive in 76 per cent; the cholesterinized antigen in 69 per cent and the Noguchi antigen in 68 per cent. This represents a difference in 8 cases of the 102 falling within this clinical group and again shows close parallelism.

We have analyzed our cases further by grouping all the initial lesions and briefly noting the duration and the result of the dark-field examination (Table IV)*.

We have also shown separately an analysis (Table II)* of the cases that did not agree in the three methods used.

In addition we have shown separately in Table III the analysis of 29 cases of syphilis that were positive with the Kolmer method and negative with the routine method using the Noguchi antigen. In this analysis it appears that 7 cases show a partial positive response with the cholesterinized antigen. It will be noted that these cases are all instances in which very considerable treatment had been given, where from 3 to 6 courses of neoarsphenamine and mercury had been administered and where, from a clinical standpoint, there was no evidence of activity. From this experience we are inclined to view Kolmer's method as being much more sensitive than the other methods employed but possibly less delicate. In other words, the Kolmer method continues positive in the face of very considerable treat-

*All tables will appear in reprints.

ment and apparent clinical cure. In the end, from a clinical standpoint, this means that the use of the Kolmer method will cause greater therapeutic effort and that a negative reaction in a known case of syphilis will have a greater diagnostic value than such a reaction with either the cholesterinized or Noguchi antigens.

It is also noteworthy that with the Kolmer method we had no anticomplementary reactions, while with our method 14 cases of the 997 examined could not be read because of anticomplementary behavior.

There still remains one serious consideration in the use of a method such as this, which in our hands has proved to be so sensitive. This concerns the attitude of the clinician toward a case of syphilis that has already received much treatment and is clinically well but still shows up very positive serologically. The persistence of a positive reaction will be discouraging both to the patient and to the physician and the question of the significance of a positive reaction in such a case is still unsettled. The possibility of overtreatment must be entertained and this applies especially to older individuals. The attitude of looking for a test to decide a clinical problem rather than permitting clinical judgment and experience to make such a decision is to be discouraged.

Furthermore, the Kolmer method is not infallible. In 19 cases of clinical syphilis (see Table II) in which our methods were positive the Kolmer method gave negative reactions.

It will be further noted that we report in Table I, 3 nonsyphilitic cases in which the Kolmer method gave a positive reaction; 5 nonsyphilitic cases in which the cholesterinized antigen was positive and 3 false positive reactions with the Noguchi antigen. These false positive reactions were regarded as possibly the result of mislabelling specimens or mishandling of materials. The probability of such accidental happenings in the handling of almost a thousand specimens must be considered. In short, we are impressed with the absence of false positive reactions when one considers the fact that in each method it represents less than a half of one per cent.

CONCLUSIONS

1. Kolmer's method of complement fixation for the diagnosis of syphilis is an excellent test.

2. It has the advantages of sensitiveness without giving false positive reactions.

3. It gives no anticomplementary reactions.

4. It has the possible disadvantage of persisting positive in clinically arrested cases.

5. From our experience we subscribe to the aim of standardization in serology. We advocate the Kolmer method as a routine test for the present.

6. However, when the aim is to study the therapeutic efficiency of syphilitic remedies we advocate a method of both complement and serum titration in the serologic procedure.

We are indebted to P. H. Carroll for very valuable technical assistance.

LABORATORY METHODS

THE GRAM-STAINING PROPERTIES OF TREPONEMA PALLIDUM*

By Dorothy Wilkes-Weiss, M.D., Philadelphia, Pa.

REVIEW OF THE LITERATURE

In searching through the literature in reference to the Gram-staining properties of Treponema pallidum, we were much disappointed at the paucity and inconclusiveness that seemed to be attached to this important phase of the organism's characteristics. With one accord, bacteriologists affirm that the Spirochaeta pallida is a very poorly staining organism and that special stains to bring out its morphology are necessary. The very fact that there are numerous methods of staining this much disputed genus indicates to the satisfaction of all that its staining qualities are, at best, slight. No mention is made by Bosanquet,[1] Calkin,[2] Stitt,[3] Rivas,[4] Muir and Ritchie,[5] or Sobernheim[6] and many others, of the applicability of the Gram's method of staining to the pallidum. Besson[7] and Park and Williams,[8] in their excellent sections on bacteriologic methods list the organism under the group of Gram-negative spirillae with no further discussion. Sobernheim[6] in his article on "Spirochetes" in Kolle and Wassermann's "Handbuch" states that "Spirochaeta pallida does not stain with the ordinary methods," and proceeds to describe about 45 varieties of staining methods for it. Among the earlier writers, in 1905, when the etiology of syphilis had just been revealed by Schaudinn and Hoffmann,[9] very few of the workers attached much importance to the attributes of the organism toward the Gram stain. Very few make mention of it with the exception of Mulzer,[10] McWeeney,[11] Muhlens[12] and Lipschütz,[13] none of these describing in any detail the method used but only emphasizing the necessity of heat while staining. It was our aim, therefore, to establish definitely the nature of the reaction of this organism to the Gram stain.

TECHNIC

It is essential that glassware of scrupulous cleanliness be used in order to rule out the presence of minute threads or specks of dirt. Glass slides and cover slips were subjected to the successive use of cleaning fluid (potassium dichromate 1 part, concentrated sulphuric acid 1 part, water 10 parts), distilled water and alcohol as a preliminary precaution.

As pointed out by Noguchi,[14] in order to apply gentian violet or similar stains to Treponema pallidum, the organism must be fixed in a properly

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buffered fixative. We have, therefore, employed his technic as follows: An equal part of the material for examination (culture media containing spirochetes, testicular emulsion harboring rabbit spirochetes or exudate from human chancre) was added to the fixative solution. This consisted of a buffered solution of formaldehyde. (One part commercial formaldehyde and nine parts phosphate buffer solution pH 7.6. The latter was prepared by adding one tablet of the required buffer [Pyrolectric Instrument Company] to 20 c.c. of distilled water under sterile precautions.) The mixture was allowed to stand, preferably in the incubator at 37½° C. for one-half to one hour. A small drop was then placed near the edge of a slide by means of a capillary pipette, spread after the manner of a blood smear and dried in the air. The staining proper resembles closely that of the usual Gram method with the exception of the fixation which was done in the above manner instead of by heat. In the author's hands, Stirling's gentian violet gave the best results, the following technic having been adhered to: The fixed and dried smear is flooded with Stirling's gentian violet which is almost immediately washed off with distilled water; Gram's iodine is allowed to act from one-half to one minute, followed by decolorization with 95 per cent alcohol, and counterstained with carbol Fuchsin (1:10). After the stained film is air dried (caution, do not blot with filter paper), it is examined under the oil immersion 1/12 objective.

EXPERIMENTAL

We applied the above technic to Treponema pallidum from various sources, hoping to establish what seems to be a much evaded characteristic of the organism. We had at our command a cultured growth of Treponema pallidum (media consisted of coagulated horse serum and bouillon), rabbits injected with Nichol's stain of Treponema pallidum, and occasionally a human chancre. Dark-field verification revealed forty to fifty spirochetes per field in our culture, four to five in the testicular suspension and two to three in the exudates from human chancres. It was found that this organism, no matter what its source, was definitely Gram-negative. There is no difficulty in staining with the Gram's method, following the details outlined, the spirochetes showing distinctly, and the various morphologic changes in those derived from cultures of varying ages being clearly manifested. Proper controls, cultures of Staphylococcus albus and of colon bacillus were similarly tested. We discovered that the Gram-staining properties of the spirochete were retained, even after five or six days' incubation with the fixative solution.

In a similar way we stained the spirillum of Vincent's angina together with various spirochetes obtained from the pockets of pyorrhea alveolaris and from the healthy mouth. These proved to be Gram-negative. An interesting fact was observed in the staining of Trypanosoma equiperdum under the above conditions; it is well known that within 10 to 15 minutes after withdrawal of blood from a mouse infected with trypanosomes, this protozoa does not retain its integrity, but quickly deteriorates, becoming granular and losing all semblance to its original morphology. The formaldehyde solution used as

fixative was found to delay this degeneration for more than an hour, the organisms showing up well and distinctly red under the influence of the counterstain.

SUMMARY

1. Review of the literature shows that with but a few exceptions, the Gram-staining property of Treponema pallidum is a neglected subject; all authors concede that this spirochete is an organism staining poorly and with difficulty at best; the Gram stain is not given as a stain applicable to the pallidum. Besson and Park and Williams mention in the list of Gram-negative organisms, Treponema pallidum, giving no details as to staining, and allowing it no place among their spirochete stains. Lipschütz in 1905 applied this stain, but succeeded only after the addition of heat.

2. Experimental evidence is given that Treponema pallidum, whether of culture, rabbit, or human origin, is easily stained and shows decolorization by Gram's method.

3. The Gram stain should be included among the various stains applicable to the staining of Treponema pallidum.

4. The formaldehyde buffer fixative solution can be used to insure against granulation and disintegration of the Trypanosoma equiperdum, when it should be found necessary to delay staining this fragile organism.

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AN IMPROVED PRECISION BLOOD-COUNTING PIPETTE*

By Carl Reich, A.B., M.D., New York, N. Y.

PRECISION pipettes have been described by May[1] and Hirschfeld.[2] The difficulties with these, however, were that they were complicated, and the blood and diluting fluid after being measured had to be mixed in a separate vessel. In this pipette it is possible to draw up the blood followed by diluting fluid just as in the ordinary pipette.

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The stop-cock has two holes so arranged that when the passage from B communicates with D, C is blocked off and *vice versa*. In operation the stop-cock is first turned so that the passage B communicates with D. Blood is now drawn up into B. It does not make any difference how far it goes up D as long as it does not pass E. The pipette is now removed from the finger, the tip wiped off and the stop-cock turned so that B communicates with C, and the precise amount of blood "a" followed by diluting fluid drawn up into bulb C up to the mark on the stem.

After the mixture has been shaken, the stop-cock is turned and a small amount of diluting fluid is drawn into E to prevent the blood in D from

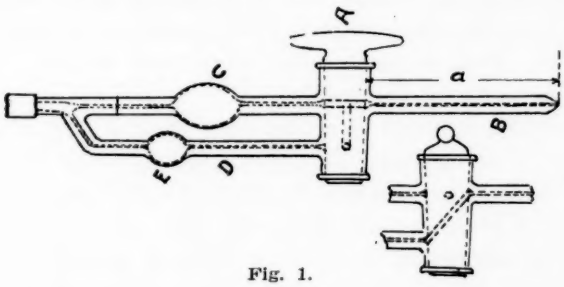

Fig. 1.

clotting. The stop-cock is now turned back so that B and C communicate and some of the mixture from C can be blown into the counting chamber.

This pipette can be adapted for red or white counts depending on the marking and the size of bulb C. It is necessary that the mark to which the blood is to be drawn whether it is 0.5 or 1.0, be right up against the stop-cock. The size of E is immaterial as it is only a little reservoir to dilute the blood in D. The diluting fluid must be stopped at its mark above the bulb, as usual, but the error involved in this case is insignificant.

This apparatus tends to increase the speed and accuracy of blood counts, for even the inexperienced operator can rapidly draw the blood up into the sidearm, turn the stop-cock and automatically get an exact column of blood for his work. This pipette is made by E. Leitz, Inc., New York.

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THE PARAFFIN SECTION METHOD*

By Robert T. Hance, Philadelphia, Pa.

THE technic of the paraffin section method as carried out in many medical schools and hospitals is unduly ponderous. The floating of paraffin sections on a basin of warm water of exactly the right temperature to cause spreading of the section without melting the surrounding paraffin and the

*From the Zoological Laboratory University of Pennsylvania.
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subsequent catching of the section on a slide is cumbersome and probably not to the best interests of the finished preparation.

An ordinary table reading lamp equipped with an arm that can be raised and lowered and a ten-inch metal shade surrounding a 150 Watt or 32 C.P. bulb serves admirably both for paraffin bath and as a source of heat for mounting paraffin sections. With this lamp on the table, over a tumbler or deep dish of paraffin the latter is melted to an inch or so from the top. By moving the lamp closer or farther away a correspondingly greater or less amount of paraffin may be melted. Tissue to be imbedded is dropped into the tumbler and will come to lie on the unmelted portion of the paraffin. Temperature control is automatic, for if the heat melts more paraffin, the tissue sinks, always remaining where the temperature is that of the melting point of paraffin. This is more satisfactory and cleaner than any of the expensive paraffin baths on the market.

To mount paraffin sections on slides with the aid of this lamp, place the albumen and water on a slide, float the sections onto this and push the slide under the lamp, which has heated the portion of the table lying directly beneath it. When a point is reached on the table where the heat is right the ribbon will slowly straighten out. This method leaves the hands free to manipulate the sections, which is particularly necessary if more than one row is to be mounted.

Mayer's albumen is, as a rule, used too generously. Two or three drops in about an ounce of water is sufficient. This does away with the somewhat messy process of smearing the slide with the albumen and then covering the whole with water. Instead the slide is flooded with this albuminized water and the sections are floated onto it. The ribbons are then spread under the lamp, the liquid drained off and the slide allowed to dry.

These methods have been worked out by Doctor C. E. McClung of the University of Pennsylvania and have been in use in his laboratories for years.

THREE HINTS ON HYDROGEN ELECTRODE TECHNIC*

By J. Percy Baumberger

1. In making platinum electrodes it is difficult to fuse a wire to the platinum foil commonly used. I have found that a piece of thin platinum wire wound into a spiral and flattened by striking while hot serves the purpose of foil in the hydrogen electrode. A deposit of platinum black is readily made on the flattened spiral and saturation with hydrogen is prompt and complete.

2. In titrating with the hydrogen electrode it is customary to make contact between the unknown solution and the calomel cell by means of saturated potassium chloride solution. The contact is made by establishing a liquid junction by allowing a glass tube of KCl to dip into the solution that

*From the Laboratory of Physiology, Stanford University.
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is being titrated. The glass tube is provided with a stop cock and the tip is bent so that diffusion will be at a minimum. A fresh contact can be made by running out some of the KCl.

Schmidt[1] has used U tubes filled with a saturated KCl solution made up to 2 per cent with washed agar-agar to make contact between the N/10 calomel electrode and the saturated KCl salt bridge. I have tried similar U tubes of KCl agar in place of the solution of KCl to make contact with the unknown in titration. The results obtained are reproducible and within

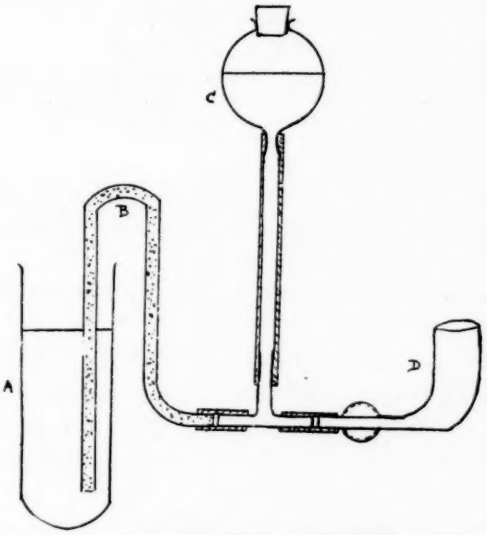

Fig. 1.—Titration vessel (*A*) connected by KCl agar-agar tube (*B*) with saturated KCl solution in *C* which is connected with calomel electrode through connecting vessel (*D*) Stippling indicates agar-agar, cross hatching indicates black rubber tubing.

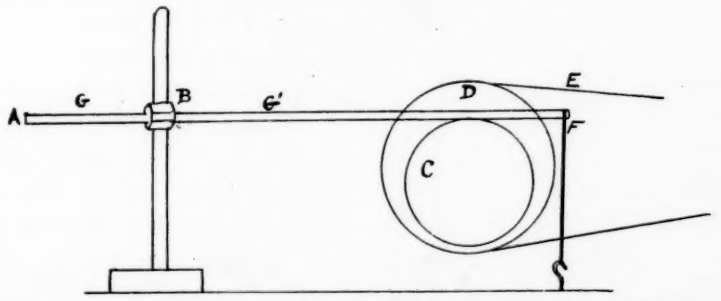

Fig. 2.—Iron rod (*A*) serves as lever arm with Swivel joint (*B*) as fulcrum and excentric (*C*) by its rotation applies force at *D*. Hydrogen ion electrode vessels may be clamped to lever at *G*, *G'*. Belt (*E*) from large pulley to motor. Rubber band (*F*) holds lever against excentric.

the limits of accuracy (3 millivolts) necessary for routine work. Fresh surfaces of agar may be exposed by cutting off a small piece of the U tube for each determination. The agar prevents the rapid diffusion usually occurring at the liquid junction of a dip contact. The discussion of potential difference at liquid junctions by Clark[2] points out possible disadvantages in the use of any but liquid junctions; however, for ordinary work an agar-liquid contact is satisfactory.

3. A simple device for rocking a Clark hydrogen electrode may be made from usual laboratory equipment by clamping one end of an iron bar 14″ long in a "clamp attachment, with swivel joint" (as it is described in supply catalogues) and resting the other end on a plain wooden pulley 2″ thick and 3½″ in diameter, this pulley being attached off center to a common slotted pulley of greater diameter. As the slotted pulley is turned by the motor the wooden pulley acts as an eccentric and the rod slides up and down with a slow even motion. Several electrodes can be clamped on the rod and by adjusting the position of the fulcrum a suitable rocking obtained.

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A MODIFICATION OF THE APPARATUS FOR DETERMINATION OF UREA BY THE UREASE METHOD*

By A. A. Horvath, V.M.B., Ph.D., Peking, China

THE determination of urea by the method described in O. Folin's "Laboratory Manual of Biological Chemistry" is rapid and gives good results, but two difficulties occur not infrequently during the process.

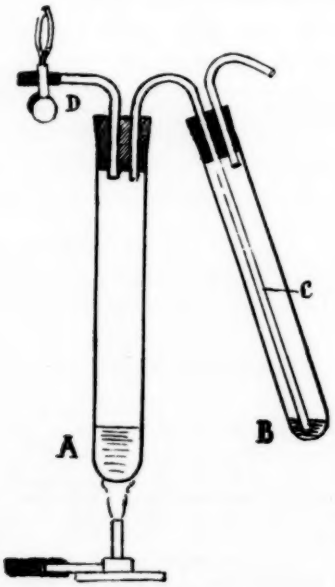

Fig. 1.

1. The formation of bubbles during boiling frequently gives rise to the danger of material being carried over into tube B (see Fig. 1).

2. Variations in the gas pressure in tube A (due to varying pressure

*From the Department of Medicine, Peking Union Medical College.
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of gas in the burner, air currents, insufficient air content in the liquid, etc.), frequently cause the liquid in B to rise in tube C or at times be carried back over into tube A.

These accidents may be avoided by the introduction of a supplementary glass tube D (as shown in the figure) which is provided with a rubber tube closed with a clamp. In instances where a large number of bubbles are formed from the liquid in A, or when there is a rise of the liquid in Tube C, or when both of those difficulties occur together, it is only necessary to open momentarily the clamp D, ordinarily without moving the burner. The atmospheric pressure will immediately break up all of the bubbles in A and replace the liquid in C. Usually the introduction of air causes the formation of bubbles to cease entirely. It gives a constant slow boiling capacity to the liquid in A and aids in keeping a satisfactory even pressure.

SUMMARY

A simple and satisfactory modification of the apparatus for determination of urea by urease has been found by introducing an additional tube with a clamp, into the tube containing the unknown, for the purpose of regulating the pressure. By this means inconvenient accidents, which occasionally occur during the distillation, were prevented.

A METHOD OF COUNTING A RAPID RHYTHMIC MOVEMENT*

By J. P. Baumberger

A RAPID rhythmic movement may be counted by tapping in unison with the contractions. If the tapping is done with the point of a lead pencil on a strip of paper for a minute, the number of dots may be counted and the rate of contraction obtained. Two students working together, one observing and tapping and the other moving the strip of paper and calling the time to start and stop, will have no difficulty. In this manner elementary students were able to count the heart beat in a Crustacean, which varies with temperature, from 100 to 400 contractions per minute.

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EDITORIALS

Rickets

RICKETS is a disease of modern civilization since it came into being with concentrations of populations in cities. It is a disease of cities.

As long as people lived active out-of-doors existences, even when, because of the inadequacies of habitations, children were forced to spend a large amount of their waking hours in the open, the disease was scarcely known; and this was true no matter how poor, within certain limits, the diet happened to be. But as centers of population grew, as housing conditions in the cities improved, as the air of cities became more and more filled with dust of one sort or another, so that the light was filtered of much of its effectiveness, rickets increased. Doubtless dietary influences were at work, too, but essentially it seems that rickets is a disease of the light that failed. This past history of the disease and the modern theories of its pathogenesis is delightfully outlined in recent reviews by Howland,[1] by Park[2] and by Hopkins.[3] As Howland says, the London of 1500 had 35,000 inhabitants, and the next largest town was York with 11,000. The housing of the people as a whole was wretched. The poorer classes lived nearly as much out of doors as within doors. In 1650 when Glisson wrote the first monograph on rickets, it was a disease of the well-to-do, whose houses were more comfortable and

in which the owners and their children spent more of their time. Since that time the incidence of the disease gradually increased, paralleling the housing improvements on the one hand and the crowding together of the poor of the temperate zone into tenements. At the present time, due to the discovery of the circumstances under which rickets appears, the disease is on the wane and there is hope of its complete disappearance.

Rickets is a serious affair, not only because of its immediate effects upon the individual, but also because of the fact that it diminishes individual resistance to other diseases, particularly those called infections. It not only stunts growth and deforms the body, but it influences adversely the efficiency of the individual and of whole populations. Its presence is a sad commentary upon modern hygiene.

The disease is characterized, chemically, by an abnormal decrease of calcium salts in the bones and it is this that accounts for the deformities, and explains in part why the disease has been looked upon as one due entirely to dietary deficiencies. It has been assumed that a diet poor in calcium was the cause of rickets, and upon this assumption a great amount of experimental work was based. Also because the calcium exists in bony tissue largely in the form of phosphates, it has been assumed that a lack of phosphorus in the diet was the cause. The fact that there might be a sufficiency of both elements in the food and that the body might not be able to absorb or to make use of it is a recent development. At any rate experimental work could not verify the hypothesis that lack of either calcium or phosphorus in the diet was an essential cause. And yet, supporting the dietary theory was the fact that cod-liver oil would prevent and cure rickets.

It was observed that experimental animals fed on certain diets would develop rickets if they were not allowed exercise and fresh air, results which gave rise to the assumption that the cause or causes of the disease lay in the surroundings of the individual rather than in his diet. One writer, v. Hausemann, stated the extreme of this view when he said that child rickets is the result of domestication, and that when man domesticated animals, he had domesticated himself. He had shut himself up in houses, he had covered his children with tight clothes and had excluded the air from them and he had gradually come to eat a refined diet, one which was unphysiologic.

Mellanby has been the main champion of the dietary causation of rickets because he was the first to produce the disease at will in experimental animals. He established a basal experimental diet that always led to the appearance of rickets, and then showed that by adding certain materials to this diet the disease could be cured or prevented. He found that cod-liver oil is superior to all other materials, and that vegetable fats were almost useless. The efficacy of cod-liver oil he laid to its richness in fat soluble vitamine A.

Opposed to Mellanby were Findley and Noel Paton who repeated Mellanby's experiments with animals that were kept in the country. These remained free from rickets even on the basal diet. Animals similarly treated, but kept in the laboratory became rachitic. They therefore held to a belief in the essential nature of exercise and fresh air. But Mellanby capped these experiments by showing that animals confined in the laboratory but with a

sufficient diet remained perfectly healthy. So whatever came out of this work, the value of cod-liver oil was proved, and this is so true that Howland says, "On any diet that will support the life of a human being or an animal, cod-liver oil will in all probability prevent or cure rickets." The same it appears might be said of sunlight.

The value of sunlight was definitely suggested by Huntly in 1889, but the first indubitable proof of its efficacy was not given until 1912. During the intervening years Huntly's remarks were lost. In 1922 Raczynski subjected to chemical examination the bodies of two puppies of the same litter, nursed by the same mother. One puppy had been kept in the sunlight, the other in the dark. The former contained more calcium and phosphate than the latter. In 1919 the value of mercury vapor lamp was proved by Huldschinsky. Following the publication of these researches both sunlight and ultraviolet radiations were used on children, and later Hess and Unger were the first to prove that sunlight alone was preventive and curative in children.

So it appears, as Howland says, that "rickets is capable of control by two masters but apparently how widely different! One is dietetic, the other environmental."

To a certain large degree rickets is prevented in infants by breast feeding. It is, as a rule, after artificial feeding commences that the disease appears, and then it begins either in closely-housed infants on good diets or in badly-fed infants poorly housed. During the dark months of the year when children must be kept protected and are therefore inactive, cod-liver oil will take the place the sunlight holds in the brighter months. Chick has shown that 90 per cent of the children she used for study, with the best food and care, showed unmistakable radiographic signs of rickets in the course of the winter and spring months, *when they were housed.* Rickets was absolutely prevented by a small amount of cod-liver oil given to the control children. When affected children were out of doors in the sun the rickets was cured rapidly. Those who were out of doors at night and not in the daytime remained unimproved.

The conditions upon which the development of rickets depends are known, but this does not mean that the cause of the disease has been discovered. Not until it is known why calcium is deposited in *any* tissue and what conditions this deposition will the cause be known. Until the physiology of calcium metabolism is known we cannot understand completely the pathology.

In rickets there is certainly a deficiency of calcium and of phosphorus in the bones and yet it is certain that many children who receive in their diets far more of both elements than can possibly be utilized contract the disease. Apparently such children make no use of them. Under the influence of cod-liver oil on the one hand and of sunlight or ultraviolet rays on the other, the state of affairs is changed and the necessary amounts of the elements are utilized.

A new aspect of the situation was developed when it was determined by blood chemical studies that the blood phosphorus was lowered in rickets, and that the blood calcium might be lowered. This is of great interest be-

cause a child receiving undiluted cow's milk may ingest nearly seven times as much phosphorus as does the breast-fed child and yet the phosphorus of the serum of the former may be only half that of the latter. Continued studies on experimental animals have shown that there are two types of rickets-producing diets, the one very low in calcium with a moderate amount of phosphorus; the other relatively low in phosphorus with a very high calcium content. On either diet rats develop rickets. But when the animals irrespective of the diet, are treated with cod-liver oil or ultraviolet radiations or sunlight, the rachitic lesions heal rapidly.

There is considerable discussion based upon theoretical assumptions as to why a sufficient supply of phosphorus and calcium in the intestinal tract is not absorbed and why, once it is absorbed, normal calcification of bones does not occur. It seems true, however, that an increase of fat in the diet facilitates absorption of both phosphorus and calcium. It appears that sunlight acts in the same way, for under either form of treatment there is a marked retention of both calcium and phosphorus.

If this be the true state of affairs, then the foundation of the disease lies in a defective absorptive power of the intestine for calcium and phosphorus. This defect is remedied by the use of certain oils or of irradiations.

The possibility remains that the ultraviolet rays modify the blood in some way and that increased absorption of the necessary materials from the intestine results.

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—*P. G. W.*

Clinical Pathology

CLINICAL pathology has developed from the routine examination of blood, urine, feces, gastric contents, etc., to a much more highly technical and exact science requiring the work of skilled individuals with adequate knowledge and experience in the various fundamental medical sciences. While well-trained technicians can perform the various procedures, few of them are qualified to efficiently carry out all the tests in each subdivision of clinical pathology such as pathology, bacteriology, immunology and biochemistry, and it is doubtful whether any have received adequate training to enable them to properly interpret all findings. For correct interpretation and particularly for authoritative application to the individual case, there can be no doubt that one who has received a general medical education is best fitted. Even such are usually not equally efficient in all of the subdivisions mentioned.

The American College of Surgeons has undertaken various lines of endeavor, not the least important among which is its work in the standardization of hospitals. The *American Society of Clinical Pathologists* has inaugurated an equally laudable program for the standardization of their portion of work in the hospitals and in clinical laboratories elsewhere. In both cases

the so-called standardization is not merely a standardization but more accurately an elevation of the existing standards. While much is to be accomplished with regard to the administration of the clinical laboratory and its relationship to the various other specialties, we feel that the Society has proceeded wisely in limiting its first endeavor as brought out in the report of its transactions, to the standardization of laboratory methods, to adjustment primarily of its own intramural activities.

The clinical pathologist is the connecting link between the laboratory and the clinician. One of the primary aims of the JOURNAL OF LABORATORY AND CLINICAL MEDICINE as has been emphasized from time to time is the exercise of a similar liason as a scientific periodical. The Society and the Journal are working with much the same objects in view. From their united endeavor the laboratory worker will find at his disposal in these pages not only the standard procedures delineated from time to time by the Society but also the most recent improvements and the new methods as they are reported by its members and by others. The clinical pathologist's interest in clinical discussions is second only to his interest in laboratory problems. The purely clinical discussions appearing in the Journal should therefore likewise be of service to him.

The Journal welcomes the opportunity to combine its endeavors with those of the American Society of Clinical Pathologists.

—*W. T. V.*

The Kolmer "Quantitative" Wassermann Reaction

WHILE the recently proposed flocculation or precipitation reactions for syphilis will probably find a definite place in serologic work, they can scarcely be expected to supercede the Wassermann reaction. The latter itself is not always conclusive, and present trends will be not so much towards simplification as towards the developing of greater reliability, even though this requires still more complicated technic.

Students of serology have long since been agreed that the original Wassermann reaction is not completely reliable. Many improvements have already been effected and several of the procedures now in use are distinctly superior to the original. A distinct drawback to the present situation is that many modifications are now in use and the results by the different methods are not always comparable. One may reply that there are various tests for albumin in the urine and that as long as they are equally accurate and reliable it should make no difference what method is utilized. Even with such a simple procedure, however, the need for standardization has been felt particularly by the insurance companies as a consequence of which a standard test for albumin has been elaborated by Burdick, Folin and Hawk, and recommended for use by the Metropolitan Life Insurance Company. A simple negative report of sputum examination for tubercle bacilli has not the value of a similar negative report, in which is included a statement as to the method of preparation and examination of the specimen and the duration of the search for acid-fast bacilli. One might say that the reporting of Wassermann

reactions would be satisfactorily adjusted were we always to indicate when reporting results, the particular technic employed. This would be an improvement but such a report would in no way increase the reliability of inferior tests. If some one method has been shown to be distinctly superior to others, that test should receive precedence.

That appreciable variability in the Wassermann results does exist is indicated by the records of observers such as Uhle and MacKinney, who submitted specimens of blood from 292 individuals to from four to ten laboratories, and found that there was but one chance in five that the tests would agree throughout; of Phelps, who in a similar study of 358 specimens, found about 65 per cent agreement; or Wolbarst who found 70 per cent agreement and 30 per cent of either slight or total disagreement; and of Palmer, who found considerable variation in 75 specimens each tested in eight different laboratories.

So many variables play a part in the Wassermann reaction that this observation is not at all surprising, particularly when we recall that the actual mechanism of the test is still unknown. Variability of antigen appears to be an important factor in causing irregularity of results. At least seven different antigens have been widely used and recommended. These are aqueous extracts of syphilitic livers, alcoholic extracts of syphilitic livers, alcoholic extracts of normal organs, alcoholic extracts of normal organs reinforced with cholesterin, acetone-insoluble lipoids, alcoholic extracts of heart reinforced with lecithin and cholesterin, and aqueous extracts of pallidum culture.

Of the many improvements, the quantitative technic recommended by Kolmer appears to have received endorsement as the most reliable so far proposed. In the elaboration of this test the author has endeavored to develop a technic: (1) of superlative sensitiveness; (2) of practical specificity; (3) of technical accuracy and uniformity of results; (4) yielding a true quantitative reaction; (5) as simple, and (6) as economical as possible. These qualifications appear in the order of their importance. The test as finally proposed has one additional advantage, the avoidance of anticomplementary reactions. Of special appeal to the clinician is the fact that the reaction will indicate more clearly the gradual reduction in strength consequent on treatment. It is often discouraging to the patient to learn that even though the therapeutic results are clear cut, the reaction still remains four plus.

Discussions of the Kolmer reaction have appeared in this Journal, particularly in the transactions of the American Society of Clinical Pathologists. The majority of authors have found the test more reliable and more sensitive than those which they had been accustomed to use and have found it entirely practicable for routine work. Even certain board of health laboratories, in which simplicity is almost as important a factor as reliability, have found it feasible to adopt this method as their standard procedure. The balance of opinion indicates that the test is the most desirable so far proposed, for acceptance as a standard technic, and that it should be so employed until increased knowledge of the mechanism of the reaction makes possible still greater refinements or simplifications, as the case may be.

—*W. T. V.*

The American Society of Clinical Pathologists

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The Third Annual Convention

"A wonderful meeting" was the universal comment of all the members and visitors who attended the third annual conclave of the American Society of Clinical Pathologists at Rochester, Minnesota. The place of the meeting under the auspices of the far-famed Mayo Clinic served undoubtedly to draw a large attendance of clinical pathologists, and their expectations were more than amply fulfilled. The diagnostic clinics and round table discussions by members of the Mayo Clinic Staff were very instructive and illuminating. Every one of the registrants came away inspired by what he had seen and stimulated to better efforts in furthering the progress of scientific medicine. The hospitality and courtesy of our hosts in throwing wide open the doors of their operating rooms, laboratories, experimental and research departments to our members elicited expressions of deep appreciation on the part of the visiting pathologists. The social features, too, in the way of entertainments for the visitors and their ladies also helped the members to carry away a pleasant recollection of a memorable meeting.

The papers read at the sessions were of a high order of excellence, reflecting the scientific spirit of our specialty and the lofty standard of the A. S. C. P.

THE STATUS OF THE CLINICAL PATHOLOGIST

The important rôle of the clinical pathologist in the present-day practice of medicine was duly emphasized by a number of speakers at our sessions representing the clinical side. The indispensable function of the pathologist as an aide to the surgeon was dwelt on by Dr. Wm. J. Mayo, who pointed out the debt that surgery owes to the exact diagnoses made in the laboratory. Operative procedures should be governed by the judicious advice rendered by the clinical pathologist as a consultant. Dr. McEachern, Executive Director of the American College of Surgeons, demonstrated the importance of the clinical pathologist in the proper organization of the Hospital and his influence in promoting standardization of institutions for the care of the sick. Other speakers regarded the clinical pathologist as the

great coordinator of the findings of the clinical and laboratory investigators, evaluating and interpreting the data thus gathered, to make the proper diagnosis and treatment. In short, at the convention, the clinical pathologist was lifted up on a plane where he seemed to be the axis on which the other medical activities revolved.

THE TRANSACTIONS

The members who were present at the meeting consider themselves fortunate in having been able to get away from their homes and spend what may be considered as a postgraduate course of instruction at the Mayo Clinic. The attendance was the largest in the history of the society, totaling over a hundred and fifty—more than half of our membership. Our members who were deprived of this pleasure will find some consolation in reading the transactions which will be published in installments in this, our official Journal. Besides the original papers read at the meeting excerpts from the round table discussions and the addresses delivered at the banquet will appear in subsequent issues.

NEWLY-ELECTED OFFICERS

The election of officers was unanimous and resulted in placing the following clinical pathologists at the head of our society for the ensuing year:

Dr. John A. Kolmer, Philadelphia, Pa., President
Dr. Frederick E. Sondern, New York, 1st Vice President
Dr. W. F. Thompson, Beaumont, Texas, 2nd Vice President
Dr. Ward Burdick, Denver, Colorado, Secretary-Treasurer

Executive Committee:
Dr. Murray G. Stone, Springfield, Mo.
Dr. Herman Spitz, Nashville, Tenn.
Dr. A. H. Sanford, Rochester, Minn.

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Dr. Ernest A. Victors, San Francisco, Cal.

The place of meeting has not been determined as yet, but will be conveniently near Atlantic City where the A. M. A. will convene in 1925.

MEMBERSHIP

Over fifty applicants for membership were voted on favorably at the business session of the convention. Included in this number are some of the leading clinical pathologists of the country. The success achieved by the A. S. C. P. in the three years of its existence, in elevating this field of medicine to its proper plane, was amply demonstrated at the Rochester meeting, judging from the encomiums bestowed on the organization, not only by members but by many clinicians and visitors at the meeting.

Admission to the Society is open to graduate physicians of good standing in their local societies who devote the major portion of their time to clinical pathology.

The objects of the A. S. C. P. are:

a. To promote the practice of scientific medicine by a wider application of clinical laboratory methods to the diagnosis of disease;

b. To stimulate original research in all branches of clinical laboratory work;

c. To establish from time to time uniform standards for the performance of various laboratory examinations;

d. To elevate the scientific and professional status of those specializing in this branch of medicine;

e. To encourage a closer cooperation between the practitioner and the clinical pathologist.

Application for membership and other information pertaining to the organization may be had from Dr. Ward Burdick, Sec'y, 652 Metropolitan Bldg., Denver, Colorado.